US-PAT-NO: 5387744
DOCUMENT-IDENTIFIER: US 5387744 A

TITLE: Avirulent microbes and uses therefor: Salmonella typhi

DATE-ISSUED: February 7, 1995

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Curtiss, III; Roy	St. Louis	MO		
Kelly; Sandra M.	St. Louis	MO		

US-CL-CURRENT: 424/258.1; 435/252.3, 435/252.33, 435/320.1, 435/879

CLAIMS:

We claim:

1. An immunogenic composition for the immunization of an individual comprising a live avirulent Salmonella having a mutation in a cdt gene said live avirulent avirulent Salmonella having the phenotype of failure to colonize deep tissue of of Salmonella deposit strain ATCC no. 55113.

2. An immunogenic composition for the immunization of an individual according to claim 1, wherein said avirulent Salmonella expresses a recombinant gene from from an agent pathogenic to said individual, to produce an antigen which induces an immune response in said vertebrate against said pathogen.

3. A method for stimulating the immune system of an individual to respond to an an immunogenic antigen of Salmonella comprising administering to said individual an immunogenic composition comprising a live avirulent Salmonella having a mutation in a cdt gene said live avirulent Salmonella having the phenotype of failure to colonize deep tissue of Salmonella deposit strain ATCC no. 55113.

4. A method for stimulating the immune system to respond to an immunogenic antigen of a pathogen comprising administering to said individual an immunogenic composition comprising a live avirulent Salmonella having a mutation in a cdt gene said live avirulent Salmonella having the phenotype of failure to colonize deep tissue of Salmonella deposit strain ATCC no. 55113.

5. A biologically pure live avirulent strain of Salmonella said live avirulent Salmonella having the phenotype of failure to colonize deep tissue of Salmonella deposit strain ATCC no. 55113.

6. The avirulent strain of Salmonella of claim 5, which expresses a recombinant gene from an agent pathogenic to said individual, to produce an antigen which induces an immune response in said vertebrate against said pathogen.

7. A strain according to claim 6, wherein the Salmonella contains a chromosomal mutation which is lethal and which is balanced by a vector-borne gene which complements the lethal mutation to constitute a balanced lethal host host vector system.

8. A strain according to claim 6, wherein cells of the strain:

a) lack a functioning native chromosomal gene encoding beta-aspartate semialdehyde dehydrogenase asd;

b) have present an exogenously introduced gene encoding a functional Asd polypeptide which phenotypically complements the chromosomal asd mutation, but which cannot replace the defective chromosomal gene by recombination; and

c) have a physical linkage between the recombinant genes encoding the functional Asd polypeptide and the immunogenic antigen, wherein the loss of the the recombinant gene encoding the functional Asd polypeptide causes the cells to lyse when the cells are in an environment in which the lack of functional Asd causes the cells to lyse.

9. A live biologically pure strain of S. typhi having a mutation in a cdt gene said live avirulent Salmonella having the phenotype of failure to colonize deep deep tissue of Salmonella deposit strain ATCC no. 55113.

10. A vaccine for the immunization of an individual comprising:

a pharmaceutically effective mount of a live avirulent Salmonella which has a diminished ability to colonize deep tissue of said individual as a result of a mutation in a cdt gene said live avirulent Salmonella having the phenotype of failure to colonize deep tissue of Salmonella deposit strain ATCC no. 55113.

11. The vaccine of claim 10 wherein said avirulent Salmonella fails to colonize colonize deep tissue of said individual. 2

First Hit Fwd Refs

L5: Entry 248 of 264 File: USPT Feb 7, 1995

US-PAT-NO: 5387744
DOCUMENT-IDENTIFIER: US 5387744 A

TITLE: Avirulent microbes and uses therefor: Salmonella typhi

DATE-ISSUED: February 7, 1995

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Curtiss, III; Roy	St. Louis	MO		
Kelly; Sandra M.	St. Louis	MO		

US-CL-CURRENT: 424/258.1; 435/252.3, 435/252.33, 435/320.1, 435/879

CLAIMS:

We claim:

1. An immunogenic composition for the immunization of an individual comprising a live avirulent Salmonella having a mutation in a cdt gene said live avirulent avirulent Salmonella having the phenotype of failure to colonize deep tissue of of Salmonella deposit strain ATCC no. 55113.

2. An immunogenic composition for the immunization of an individual according to claim 1, wherein said avirulent Salmonella expresses a recombinant gene from from an agent pathogenic to said individual, to produce an antigen which induces an immune response in said vertebrate against said pathogen.

3. A method for stimulating the immune system of an individual to respond to an an immunogenic antigen of Salmonella comprising administering to said individual an immunogenic composition comprising a live avirulent Salmonella having a mutation in a cdt gene said live avirulent Salmonella having the phenotype of failure to colonize deep tissue of Salmonella deposit strain ATCC no. 55113.

4. A method for stimulating the immune system to respond to an immunogenic antigen of a pathogen, comprising administering to said individual an immunogenic composition comprising a live avirulent Salmonella having a mutation in a cdt gene said live avirulent Salmonella having the phenotype of failure to colonize deep tissue of Salmonella deposit strain ATCC no. 55113.

5. A biologically pure live avirulent strain of Salmonella said live avirulent Salmonella having the phenotype of failure to colonize deep tissue of Salmonella deposit strain ATCC no. 55113.

6. The avirulent strain of Salmonella of claim 5, which expresses a recombinant recombinant gene from an agent pathogenic to said individual, to produce an antigen which induces an immune response in said vertebrate against said pathogen.

7. A strain according to claim 6, wherein the Salmonella contains a chromosomal chromosomal mutation which is lethal and which is balanced by a vector-borne gene which complements the lethal mutation to constitute a balanced lethal host host vector system.

8. A strain according to claim 6, wherein cells of the strain:

a) lack a functioning native chromosomal gene encoding beta-aspartate semialdehyde dehydrogenase asd;

b) have present an exogenously introduced gene encoding a functional Asd polypeptide which phenotypically complements the chromosomal asd mutation, but which cannot replace the defective chromosomal gene by recombination; and

c) have a physical linkage between the recombinant genes encoding the functional Asd polypeptide and the immunogenic antigen, wherein the loss of the the recombinant gene encoding the functional Asd polypeptide causes the cells to lyse when the cells are in an environment in which the lack of functional Asd causes the cells to lyse.

9. A live biologically pure strain of S. typhi having a mutation in a cdt gene said live avirulent Salmonella having the phenotype of failure to colonize deep deep tissue of Salmonella deposit strain ATCC no. 55113.

10. A vaccine for the immunization of an individual comprising:

a pharmaceutically effective mount of a live avirulent Salmonella which has a diminished ability to colonize deep tissue of said individual as a result of a mutation in a cdt gene said live avirulent Salmonella having the phenotype of failure to colonize deep tissue of Salmonella deposit strain ATCC no. 55113.

11. The vaccine of claim 10 wherein said avirulent Salmonella fails to colonize colonize deep tissue of said individual. 2

First Hit Fwd Refs

L5: Entry 211 of 264 File: USPT Jan 5, 1999

US-PAT-NO: 5855879
DOCUMENT-IDENTIFIER: US 5855879 A

TITLE: Avirulent microbes and uses therefor

DATE-ISSUED: January 5, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Curtiss III; Roy	St. Louis	MO		

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Washington University	St. Louis	MO			02

APPL-NO: 08/ 209542 [PALM]
DATE FILED: March 10, 1994

PARENT-CASE:
CROSS-REFERENCE TO RELATED APPLICATIONS This application is a divisional of application Ser. No. 07/785,748, filed Nov. 7, 1991, now U.S. Pat. No. 5,294,441, which is a continuation-in-part of U.S. application Ser. No. 07/612,001, filed Nov. 9, 1990, now abandoned; which is a continuation in part of U.S. application Ser. No. 07/200,934, filed Jun. 1, 1988, now abandoned, which is a continuation-in-part of U.S. application Ser. No. 07/058,360, filed Jun. 4, 1987, now abandoned; U.S. application Ser. No. 07/612,001 is also a continuation-in-part of U.S. application Ser. No. 07/251,304, filed Oct. 3, 1988, now abandoned, which is a continuation-in-part of U.S. application Ser. No. 07/106,072, filed Oct. 7, 1987, now abandoned. These applications are hereby incorporated herein by reference.

INT-CL: [06] A61 K 39/02, A61 K 39/112, C12 N 1/21

US-CL-ISSUED: 424/952; 424/93.48, 424/184.1, 424/200.1, 424/235.1, 424/257.1, 424/258.1, 435/172.3, 435/252.3, 435/252.33, 435/320.1, 435/879
US-CL-CURRENT: 424/93.2; 424/184.1, 424/200.1, 424/235.1, 424/257.1, 424/258.1, 424/93.48, 435/252.3, 435/252.33, 435/320.1, 435/879

FIELD-OF-SEARCH: 435/172.3, 435/320.1, 435/252.3, 435/252.33, 435/879, 424/93.2, 424/93.48, 424/184.1, 424/200.1, 424/235.1, 424/258.1, 424/257.1

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

Search Selected Search ALL Clear

PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL

☐	4190495	February 1980	Curtis	435/172.3
☐	4837151	June 1989	Stocker	435/172.3

OTHER PUBLICATIONS

Komeda et al. Molec. Gen. Genet. vol. 142, 289-298 (1975).
Ferrari et al. Biotechnology, vol. 3, Nov. 1985, pp. 1003-1007.
Jagusztyn-Krynicka et al., Journal of General Microbiology (1982) pp. 1135-1145.

ART-UNIT: 188

PRIMARY-EXAMINER: Scheiner; Laurie

ATTY-AGENT-FIRM: Howell & Haferkamp, L.C.

ABSTRACT:

This invention provides immunogenic compositions for the immunization of a vertebrate or invertebrate comprising an avirulent derivative of S. typhi. The derivatives having a mutation of the cya and/or crp and/or cdt genes. The invention also provides immunogenic compositions for the immunization of a vertebrate and invertebrate comprising an avirulent derivative of the above type which is capable of expressing a recombinant gene derived from a pathogen of said vertebrate or invertebrate individual to produce an antigen capable of inducing an immune response response against said pathogen. Other embodiments of the invention include methods of preparing immunogenic compositions from these strains, and strains useful in the preparation of the immunogenic compositions, as well as methods of stimulating the immune system to respond to an immunogenic antigen of S. typhi by administration of the immunogenic composition.

9 Claims, 6 Drawing figures

DOCUMENT-IDENTIFIER: US 5855879 A
TITLE: Avirulent microbes and uses therefor

Abstract Text (1):
This invention provides immunogenic compositions for the immunization of a vertebrate or invertebrate comprising an avirulent derivative of S. typhi. The derivatives having a mutation of the cya and/or crp and/or cdt genes. The invention also provides immunogenic compositions for the immunization of a vertebrate and invertebrate comprising an avirulent derivative of the above type which is capable of expressing a recombinant gene derived from a pathogen of said vertebrate or invertebrate individual to produce an antigen capable of inducing an immune response against said pathogen. Other embodiments of the invention include methods of preparing immunogenic compositions from these strains, and strains useful in the preparation of the immunogenic compositions, as well as methods of stimulating the immune system to respond to an immunogenic antigen of S. typhi by administration of the immunogenic composition.

Brief Summary Text (2):
This invention relates to avirulent microbes, their method of preparation, and their use in vaccines.

Brief Summary Text (4):
Typhoid fever, which is caused by Salmonella typhi, remains an important public health problem for residents in the less developed world, for travelers from industrialized countries who visit endemic areas, and for clinical microbiologists in laboratories which conduct proficiency tests. The currently licensed parenteral killed whole cell typhoid vaccines are protective but cause marked systemic and local adverse reactions at an unacceptably high frequency (Levine, Typhoid fever vaccines, in Plotkin S. A., Mortimer E. A. Jr. (eds): VACCINES. Philadelphia, W. B. Saunders, 1988, pp. 333-361). Alternative vaccines include the recently licensed live oral vaccine strain Ty21a and the experimental parenteral Vi polysaccharide vaccine.

Brief Summary Text (5):
The advantage of an oral vaccine is the delivery of replicating organisms to the mucosal immune system where local responses are maximally stimulated. In addition, attenuated Salmonella typhi are attractive candidates to serve as carrier vaccines to express foreign antigens and deliver them to the human immune system. However, a critical prerequisite for successfully using this approach in immunizing humans is that there must exist highly immunogenic yet safe attenuated strains of S. typhi to deliver the foreign protein and polysaccharide antigens to the immune system.

Brief Summary Text (6):
The current oral vaccine based upon Ty21a has several disadvantages. Ty21a is of relatively low immunogenicity and requires multiple oral doses to immunize. The yield of viable organisms is low when it is fermented and lyophilized in large-scale. In addition, Ty21a has multiple mutations in addition to galE and via, which remain undefined. (Hone et al. (1987), J. Infect. Dis. 156:167-174; Hone et al. (1988), J. Infect. Immun. 56:1326-1333).

Brief Summary Text (7):
Constructs of Ty21a expressing the O antigen of Shigella sonnei (Formal et al. (1981), Infect. Immun. 34:746-750) or the O antigen of Vibrio cholerae 01 serotype Inaba (Forrest et al. (1989), J. Infect. Dis. 159:145-146) have undergone clinical testing in humans. Although two lots of the Ty21a/S. sonnei construct tested in North American volunteers provided significant protection against experimental challenge with pathogenic S. sonnei, there was lot-to-lot variation and other lots were not protective (Black et al. J. Infect. Dis. (1987), 155:1260-1267; Herrington et al. (1990), Vaccine 8:353-357). The

Ty21a/Inaba construct elicited serum Inaba vibriocidal antibodies and intestinal SIgA anti-Inaba O antibodies in only a minority of vaccines and at low titer (Tacket et al. (1990), Infect. Immun. 1620-1627). In experimental challenge studies with pathogenic V. cholerae 01, recipients of the construct were not significantly protected overall against diarrhea, but did have milder diarrhea and shed fewer wild-type V. cholerae cells (Tacket et al., Id.).

Brief Summary Text (9):
Applicant has discovered new methods of protecting against virulent infections with vaccines employing transposon-induced avirulent mutants of virulent agents in which the impairment leading to avirulence cannot be repaired by diet or by anything supplied by an animal host. Some of Applicant's initial work, including a method for creating an avirulent microbe by the introduction of deletion mutations in the adenylate cyclase gene (cya) and the cyclic AMP receptor protein gene (crp) of Salmonella typhimurium is described in EPO Pub. No. 315,682 (published 17 May 1989), and PCT Pub. No. WO 88/09669 (published 15 Dec. 1988). Applicant has also provided methods for producing other types of avirulent mutant cells which are desirable as carrier cells for the expression of recombinant antigens. These cells are characterized by a lack of a functioning native gene encoding an enzyme which is essential for cell survival, wherein the enzyme catalyses a step in the biosynthesis of an essential cell wall structural component and the presence of a first recombinant gene encoding an enzyme which is a functional replacement for the native enzyme, wherein the first recombinant gene cannot replace the defective chromosomal gene. In these cells, the first recombinant gene is structurally linked to a second recombinant gene encoding a desired product. Loss of the first recombinant gene causes the cells to lyse. These methods are described in Wo 89/03427 (published 20 Apr. 1989). The disclosures of the above-described patent applications, as well as any corresponding national patent applications, are incorporated herein by reference.

Brief Summary Text (11):
The present invention is based, in part, on new avirulent S. typhi derivatives that are not disclosed in EPO Pub. No. 315,682. Included within the invention is the application of these new S. typhi derivatives in, inter alia, commercial vaccines, methods of stimulating the immune system to respond to an immunogenic antigen of S. typhi, and methods of stimulating the immune system to respond to an immunogenic antigen of a pathogen. The strains provided herein are directly and indirectly suitable for the production of commercial vaccines to prevent diseases caused by S. typhi, and other enteric bacteria with which antibodies to S. typhi cross react. These strains are also useful as carrier microorganisms for the production of expression products encoded on recombinant genes in the bacterial cells.

Detailed Description Text (2):
This invention is predicated on the discovery that certain mutations can render a microbe avirulent without substantially affecting its immunogenicity. More specifically, this invention relates to microbial vaccines in which the microbe carries the deletion (open triangle or delta) mutations .DELTA.cya and/or .DELTA.crp eliminating the ability to synthesize adenylate cyclase (ATP pyrophosphate lyase (cyclizing) EC 4.6.1.1) and the cyclic AMP receptor protein (CRP), respectively.

Detailed Description Text (5):
Once rendered avirulent by the introduction of the .DELTA.cya and/or .DELTA.crp mutations, the microbes can serve as the immunogenic component of a vaccine to induce immunity against the microbe.

Detailed Description Text (7):
In still another embodiment of the invention, the avirulent derivative of a pathogenic S. typhi can be used as a carrier bacteria to deliver selected antigens to the GALT, for example to the Peyer's patches of the ileum. Salmonella are known to home to the Peyer's patches (Carter, P. B. and F. M. Collins, J. Exp.

Med. 139:1189 (1974)). S. typhimurium-E. coli hybrids have also been shown to colonize Peyer's patches in mice (Hohmann, A. W., et al., Infect. and immun. 22:763 (1978)). If these carrier bacteria contain and express a recombinant gene from a pathogenic organism, antibodies against the antigenic gene product produced from the pathogen will be induced. With the advent of recombinant DNA techniques, it now becomes possible to develop totally unique vaccines in which specific antigens are produced, not by the etiologic agent, but by another host strain of bacteria capable of expressing the gene for that antigen. It is also possible, when antigens might cross-react with an antigen of the mammalian host and thus potentiate the induction of autoimmunity, to use recombinant DNA techniques to alter the gene so that the affecting cross-reacting antigenic determinant is not produced. Thus, recombinant DNA techniques can be employed to develop vaccines that do not have any material capable of cross-reacting with vertebrate host antigens or capable to eliciting an autoimmune state.

Detailed Description Text (10):
It is apparent that the present invention has wide applicability to the development of effective vaccines against bacterial, fungal, parasite or viral disease agents where local immunity is important and might be a first line of defense. Some examples are vaccines for the control of pneumonic plague caused by Yersinia pestis, of gonorrhea caused by Neisseria gonorrhoeae, of syphilis caused by Treponema pallidum, and of venereal diseases as well as eye infections caused by Chlamydia trachomatis. Species of Streptococci from both group A and group B, such as those species that cause sore throat or heart disease, Neisseria meningitidis, Mycoplasma pneumoniae, Haemophilus influenzae, Bordetella pertussis, Mycobacterium tuberculosis, Mycobacterium leprae, Bordetella avium, Escherichia coli, Streptococcus equi, Streptococcus pneumoniae, Brucella abortus, Pasteurella hemolytica, Vibrio cholerae, Shigella species, and Legionella pneumophila are additional examples of bacteria within the scope of this invention from which genes could be obtained. Viral vaccines, such as those produced against influenza viruses, are also encompassed by this invention. Viral vaccines can also be produced against other viruses, either DNA or RNA viruses, for example from the classes Papovirus, Adenovirus, Herpesvirus, Poxvirus, Parvovirus, Reovirus, Picornavirus, Myxovirus, Paramyxovirus, or Retrovirus. Vaccines to protect against infection by pathogenic fungi, protozoa and parasites are also contemplated by this invention.

Detailed Description Text (11):
In a further embodiment, when the immunogenic component of the vaccine is an allergen of the host such a vaccine may be used in an exposure regimen designed to specifically desensitize an allergic host.

Detailed Description Text (12):
In one of its embodiments, the idesction can be described as a vaccine for the immunization of a vertebrate animal or invertebrate comprising a live avirulent derivative of a pathogenic microbe said derivative being incapable of producing functional adenylate cyclase and cAMP receptor protein while being capable of expressing a recombinant gene derived from an organism that is a pathogen of or that produces an allergen of said animal.

Detailed Description Text (13):
In yet another embodiment the avirulent microbes of this invention may be used as vectors for the synthesis of various host proteins. Because the avirulent microbes of this invention are able to traverse a variety of immunocompetent structures including GALT, mesenteric lymph nodes and spleen after introduction into the host, such microbes may be used to target a variety of immunoregulatory products. Accordingly, one or more genes encoding immunoregulatory proteins or peptides may be recombinantly introduced into the avirulent microbes such that when the microbes taking up residence in the appropriate immunocompetent tissue are capable of expressing the recombinant product to suppress, augment or modify the immune response in the host. Examples of immunoregulatory molecules include but are not limited to: colony stimulating factors (macrophage, granulocyte, or mixed), macrophage

chemotoxin, macrophage inhibition factor, leukocyte inhibitory factors, lymphotoxins, blastogenic factor, interferon, and interleukins.

Detailed Description Text (17):
By immunogenic agent is meant an agent used to stimulate the immune system of a living organism, so that one or more functions of the immune system are increased and directed towards the immunogenic agent. Immunogenic agents include vaccines. Immunogenic agents can be used in the production of antibodies, both isolated polyclonal antibodies and monoclonal antibodies, using techniques known in the art.

Detailed Description Text (18):
By vaccine is meant an agent used to stimulate the immune system of a living organism so that protection against future harm is provided. Immunization refers to the process of inducing a continuing high level of antibody and/or cellular immune response in which T-lymphocytes can either kill the pathogen and/or activate other cells (e.g., phagocytes) to do so in an organism, which is directed against a pathogen or antigen to which the organism has been previously exposed. Although the phrase "immune system" can encompass responses of unicellular organisms to the presence of foreign bodies, e.g., interferon production, in this application the phrase is restricted to the anatomical features and mechanisms by which a multi-cellular organism produces antibodies against an antigenic material which invades the cells of the organism or the extra-cellular fluid of the organism. The antibody so produced may belong to any of the immunological classes, such as immunoglobulins A, D, E, G or M. Of particular interest are vaccines which stimulate production of immunoglobulin A (IgA) since this is the principle immunoglobulin produced by the secretory system of warm-blooded animals, although vaccines of the invention are not limited to those which stimulate IgA production. For example, vaccines of the nature described herein are likely to produce a broad range of other immune responses in addition to IgA formation, for example, cellular and humoral immunity. Immune response to antigens is well studied and widely reported. A survey of immunology is given in Barrett, James, T., Textbook of Immunology: Fourth Edition, C. V. Mosby Co., St. Louis, Mo. (1983), the entire of which is herein incorporated by reference.

Detailed Description Text (19):
A vertebrate is any member of the subphylum Vertebrata, a primary division of the phylum Chordata that includes the fishes, amphibians, reptiles, birds, and mammals, all of which are characterized by a segmented bony or cartilaginous spinal column. All vertebrates have a functional immune system and respond to antigens by producing antibodies. Thus, all vertebrates are capable of responding to vaccines. Although vaccines are most commonly given to mammals, such as humans or dogs (rabies vaccine), vaccines for commercially raised vertebrates of other classes, such as the fishes and birds if of the nature described herein, are within the scope of the present invention.

Detailed Description Text (20):
An invertebrate is any member of the Animal Kingdom, excluding the vertebrates. Such animals constitute the Division Invertebrata and have no backbone or spinal column. This classification includes all animals except fishes, amphibians, reptiles, birds and mammals. Many invertebrates are capable of illiciting a primitive immune response to antigenic stimulation and are susceptible to the same microorganisms which infect vertebrates and which are disclosed herein in accordance with this invention. Exemplary of such invertebrates are shellfish and molluses and other related animals. Although the use of vaccines in the protection of invertebrate animals have hitherto before not been well documented, one skilled in the art will recognize the applicability of the subject invention to said invertebrates by use of their primitive immune systems. For example, and in accordance with this invention, the susceptibility of shellfish to infection by Salmonella will allow the introduction of avirulent strains of Salmonella species and thereby provide potential for the primitive immune system to

respond. Therefore, it is within the scope of this invention, the use of an avirulent derivative of a pathogenic microbe, that is capable of infecting an invertebrate, to stimulate a response from an immune system present in said invertebrate against a pathogen.

Detailed Description Text (21):
An "individual" treated with a vaccine of the invention is defined herein as including all vertebrates, for example, mammals, including domestic animals and humans, various species of birds, including domestic birds, particularly those of agricultural importance. In addition, mollusks and certain other invertebrates have a primitive immune system, and are included as an "individual".

Detailed Description Text (24):
In order for the gene to be effective in eliciting an immune response, the gene must be expressed. Expression of a gene means that the information inherent in the structure of the gene (the sequence of DNA bases) is transformed into a physical product in the form of an RNA molecule, polypeptide or other biological molecule by the biochemical mechanisms of the cell in which the gene is located. The biological molecule so produced is called the gene product. The term gene product as used here refers to any biological product or products produced as a result of the biochemical reactions that occur under the control of a gene. The gene product may be, for example, an RNA molecule, a peptide, or a product produced under the control of an enzyme or other molecule that is the initial product of the gene, i.e., a metabolic product. For example, a gene may first control the synthesis of an RNA molecule which is translated by the action of ribosomes into an enzyme which controls the formation of glycans in the environment external to the original cell in which the gene was found. The RNA molecule, the enzyme, and the glycan are all gene products as the term is used here. Any of these as well as many other types of gene products, such as glycoproteins and polysaccharides, will act as antigens if introduced into the immune system of an animal. Protein gene products, including glycoproteins and lipoproteins, are preferred gene products for use as antigens in vaccines.

Detailed Description Text (25):
In order for a vaccine to be effective in producing antibodies, the antigenic material must be released in such a way that the antibody-producing mechanism of the vaccinated animal can come into play. Therefore, the microbe carrier of the gene product must be introduced into the animal. In order to stimulate a preferred response of the GALT or BALT cells as discussed previously, introduction of the microbe or gene product directly into the gut or bronchus is preferred, such as by oral administration, gastric intubation or in the form of aerosols, although other methods of administering the vaccine, such as intravenous, intramuscular, subcutaneous injection or intramammary or intrapenial or vaginal administration, are possible.

Detailed Description Text (26):
When the avirulent microbe is used as a carrier microbe, and once the carrier microbe is present in the animal, the antigen needs to become available to the animal's immune system. This may be accomplished when the carrier microbe dies so that the antigen molecules are released. Of course, the use of "leaky" avirulent mutants that release the contents of the periplasm without lysis is also possible. Alternatively, a gene may be selected that controls the production of an antigen that will be made available by the carrier cell to the outside environment prior to the death of the cell. In this way, it is possible to use a viable microbe that will persist in the vaccinated animal, for example in its Peyer's patches, and continue to produce antigen, thereby continually inducing antibody formation. A preferred gene product under these circumstances is a product that is transferred through the cell membrane into the external environment or a product that becomes attached to or embedded in the external membrane so that all or part of the gene product is exposed to the environment. Typical of this latter type of gene product are antigens normally found on the surface of the organism against which protection is desired. If these antigens are transported to the cell surface in a normal manner, antibody formation against the

antigens will be enhanced.

Detailed Description Text (29):
Administration of a live vaccine of the type disclosed above to an animal may be by any known or standard technique. These include oral ingestion, gastric intubation, or broncho-nasal-ocular spraying. All of these methods allow the live vaccine to easily reach the GALT or BALT cells and induce antibody formation and are the preferred methods of administration. Other methods of administration, such as intravenous injection, that allow the carrier microbe to reach the animal's blood stream may be acceptable. Intravenous, intramuscular or intramammary injection are also acceptable with other embodiments of the invention, as is described later.

Detailed Description Text (30):
Since preferred methods of administration are oral ingestion, aerosol spray and gastric intubation, preferred carrier microbes are those that belong to species that attach to, invade and persist in any of the lymphoepithelial structures of the intestines or of the bronchii of the animal being vaccinated. These strains are preferred to be avirulent derivatives of enteropathogenic strains produced by genetic manipulation of enteropathogenic strains. Strains that attach to, invade and persist in Peyer's patches and thus directly stimulate production of IgA are most preferred. In animals these include specific strains of Salmonella, and Salmonella-E. coli hybrids that home to the Peyer's patches.

Detailed Description Text (34):
Derivatives of avirulent microbes are also contemplated to be within the scope of this invention. By derivative is meant sexually or asexually derived progeny and mutants of the avirulent strains including single or multiple base substitutions, deletions, insertions or inversions which retain the inability to produce functional adenylate cyclase and/or cAMP receptor protein and/or the expression of the cdt gene, with or without naturally occurring virulence plasmids. For example, strains such as 4062 and 4064 carry the gyrA mutation conferring nalidixic acid resistance which has been used herein as a convenient marker to follow strains through the animal following oral inoculation. However, drug resistance is not a desirable attribute for strains to be used as vaccines. Thus, the gyrA mutation can be easily removed by transducing the wild-type gyrA+ (conferring sensitivity to nalidixic acid) gene into strains by selecting for inheritance of a closely linked Tn10 and then removing Tn10 by transduction with a phage lysate propagated on the parent strain carrying the gyrA.sup.- allele with selection for fusaric acid resistance.

Detailed Description Text (35):
The dosages required will vary with the antigenicity of the gene product and need only be an amount sufficient to induce an immune response typical of existing vaccines. Routine experimentation will easily establish the required amount. Multiple dosages are used as needed to provide the desired level of protection.

Detailed Description Text (36):
The pharmaceutical carrier or excipient in which the vaccine is suspended or dissolved may be any solvent or solid or encapsulated in a material that is non-toxic to the inoculated animal and compatible with the carrier organism or antigenic gene product. Suitable pharmaceutical carriers are known in the art, and for example, include liquid carriers, such as normal saline and other non-toxic salts at or near physiological concentrations, and solid carriers, such as talc or sucrose and which can also be incorporated into feed for farm animals. Adjuvants may be added to enhance the antigenicity if desired. When used for administering via the bronchial tubes, the vaccine is preferably presented in the form of an aerosol. Suitable pharmaceutical carriers and adjuvants and the preparation of dosage forms are described in, for example, Remington's Pharmaceutical Sciences, 17th Edition, (Gennaro, Ed., Mack Publishing Co., Easton, Pa., 1985).

Detailed Description Text (37):
Immunization with a pathogen-derived gene product can also be used in conjunction with prior immunization with the avirulent derivative of a pathogenic microorganism acting as a carrier to express the gene product specified by a recombinant gene from a pathogen. Such parenteral immunization can serve as a booster to enhance expression of the secretory immune response once the secretory immune system to that pathogen-derived gene product has been primed by immunization with the carrier microbe expressing the pathogen-derived gene product to stimulate the lymphoid cells of the GALT or BALT. The enhanced response is known as a secondary, booster, or anamnestic response and results in prolonged immune protection of the host. Booster immunizations may be repeated numerous times with beneficial results.

Detailed Description Text (53):
Genetic stability of avirulent mutants. Strains to be orally administered as live vaccines must have complete stability with regard to both their avirulence and their immunogenic attributes. When 50-fold concentrated cultures and various dilutions (.about.10.sup.9, 10.sup.7, 10.sup.5, 10.sup.3 CFU/plate) of each of the ten independent .DELTA.cya mutants and each of the ten independent .DELTA.crp mutants were plated on minimal agar media (supplemented with 22 .mu.g cysteine/ml and 22 .mu.g arginine/ml) containing 0.5% maltose, melibiose, xylose, glycerol, or rhamnose that should not support their growth, revertants and mutants were not detected. One set of duplicate plates were UV-irradiated (5 joules/meter.sup.2 /sec) and incubated at 37.degree. C. in the dark. The other set of plates was incubated at 37.degree. C. with illumination. Revertants and mutants were not detected after a 48 h growth period. An investigation was also conducted as to whether tetracycline-resistant revertants/mutants could be recovered from the fusaric acid resistant .DELTA.cya and .DELTA.crp mutants at frequencies higher than could be observed for the tetracycline-sensitive wild-type parental strain. In all cases, such tetracycline-resistant revertants/mutants were not observed.

Detailed Description Text (62):
Construction of S. tyohimurium strains with .DELTA.cya-12 and .DELTA.crp-11 deletion mutations. The best vaccine strains in terms of efficacy are likely to result from the attenuation of highly virulent strains that display significant colonizing ability and invasiveness. The criteria for selection of these highly pathogenic S. typhimurium wild-type strains such as SL1344 (.chi.3339), UK-1 (.chi.3761) and 798 included low LD.sub.50 values (see Table 4) in mouse virulence assays, antibiotic sensitivity, possession of the virulence plasmid, ease of genetic manipulation (bacteriophage P22HTint or P1 sensitivity, transformability and ease of receiving mobilized plasmids), and colicin sensitivity.

Detailed Description Text (68):
Genotypic and phenotypic stability of avirulent mutants. Methods for determining stability of genetic traits are as described in Example 1. All genotypic and phenotypic traits due to the .DELTA.cya .DELTA.crp mutations were completely stable except motility. Although synthesis of functional flagella and display of motility is dependent on wild-type cya and crp gene functions, a suppressor mutation in the cfs (constitutive flagellar synthesis) gene can easily be selected to cause flagella synthesis and motility to be independent of cya and crp gene functions. In S. typhimurium .DELTA.cya .DELTA.crp strains, motile variants were readily selected during the strain construction process. Since immunity to flagellar antigens may be protective, motile variants of all vaccine strains were selected.

Detailed Description Text (92):
It is evident that inclusion of the .DELTA.[crp-cysG]-10 or .DELTA.[crp-cysG]-14 mutations which are also .DELTA.cdt mutations would enhance the safety of live attenuated Salmonella vaccine strains while not diminishing their immunogenicity. This might be particularly important for host-adapted

invasive Salmonella species such as S. typhi, S. paratyphi A (S. schottmuelleri), S. Paratyphi B (S. hirshfeldii), S. paratyphi C (all infect humans), S. choleraesuis (infects swine), S. dublin (infects cattle), S. gallinarum, and S. pullorum (both infect poultry), as well as non-host specific, invasive Salmonella species such as S. typhimurium and S. enteritidis.

Detailed Description Text (98):
Construction of S. typhimurium strains with .DELTA.cya-12 and .DELTA.[crp-cysG]-10 deletion mutations. The best vaccine strains in terms of efficacy are likely to result from the attenuation of highly virulent strains that display significant colonizing ability and invasiveness. The criteria for selection of these highly pathogenic S. typhimurium wild-type strains such as SL1344 (.chi.3339), UK-1 (.chi.3761) and 798 has been described in Example 2.

Detailed Description Text (104):
Genotypic and phenotypic stability of avirulent mutants. Methods for determining stability of genetic traits are as described in Example 1. All genotypic and phenotypic traits due to the .DELTA.cya .DELTA.crp mutations were completely stable except motility. Although synthesis of functional flagella and display of motility is dependent on wild-type cya and crp gene functions, a suppressor mutation in the cfs (constitutive flagellar synthesis) gene can easily be selected to cause flagella synthesis and motility to be independent of cya and crp gene functions. In S. typhimurium .DELTA.cya .DELTA.crp strains, motile variants were readily selected during the strain construction process. Since immunity to flagellar antigens may be protective, motile variants of all vaccine strains were selected.

Detailed Description Text (117):
Construction of S. typhi strains with cya and crp mutations. The wild-type, virulent S. typhi Ty2 (type E1), ISP1820 (type 46) and ISP2822 (type E1) strains were genetically modified as described below, using classical genetic methods similar to those described in Curtiss and Kelly (1987). ISP1820 and ISP2822 were recently isolated during a typhoid epidemic in Chile and are likely to be more invasive than the standard laboratory Ty2 strain of S. typhi. Their attenuation might therefore generate vaccine strains that would be more efficacious than those derived from Ty2. The construction strategy consists of mobilizing deletions of crp and cya genes that have been isolated and characterized in S. typhimurium SL1344 by placing the transposon Tn10 (encoding tetracycline resistance) nearby the .DELTA.cya or .DELTA.crp mutation and transducing the linked traits into the highly virulent S. typhi Ty2, ISP1820 and ISP2822 strains via P22HTint-mediated transduction with selection for tetracycline resistance and screening for a maltose-negative phenotype. The zhc-1431::Tn10 linked to crp and zid-62::Tn10 linked to cya were used for this purpose. Neither insertion alone affects virulence of S. typhimurium.

Detailed Description Text (123):
Genetic stability of avirulent mutants. Strains to be orally administered as live vaccines must have complete stability with regard to their avirulence attributes. When 50-fold concentrated cultures and various dilutions (.about.10.sup.9, 10.sup.7, 10.sup.5, 10.sup.3 CFU/plate) of the .DELTA.cya .DELTA.crp S. typhi strains were plated on minimal agar media (supplemented with required amino acids) containing 0.5% maltose, melibiose, xylose, glycerol, or rhamnose that should not support their growth, revertants and mutants were not detected. One set of duplicate plates was UV-irradiated (5 joules/meter.sup.2 /sec) and incubated at 37.degree. C. in the dark. The other set of plates was incubated at 37.degree. C. with illumination. Revertants and mutants were not detected after a 48 h growth period. An investigation was also conducted as to whether tetracycline-resistant revertants/mutants could be recovered at frequencies higher than could be observed for the parental strain. In all cases, such tetracycline-resistant revertants/mutants were not observed.

Detailed Description Text (124):

Virulence of mutant strains for mice. Table 9 contains data which show that mice survive infection with about 10.sup.4 times the LD.sub.50 dose of either .chi.3926 or .chi.3927. The natural host for S. typhi is man. Therefore, hog gastric mucin is used as a virulence enhancer of S. typhi cells in mice, and thus maximizes the virulence of S. typhi vaccine candidates in this model system.

Detailed Description Text (132):
The wild-type, virulent Ty2 (type E1), ISP1820 (type 46) and ISP2822(type E1) strains were genetically modified as described below, using classical genetic methods similar to those described in Curtiss and Kelly (1987). ISP1820 and ISP2822 were recently isolated during a typhoid epidemic in Chile and are likely to be more invasive than the standard laboratory Ty2 strain of S. typhi. Their attenuation might therefore generate vaccine strains that could be more efficacious than those derived from Ty2. The construction strategy consists of mobilizing deletions of crp and cya genes that have been isolated and characterized in S. typhimurium SL1344 (as described in Example 1) by placing the transposon Tn10 (encoding tetracycline resistance) nearby the .DELTA.cya or .DELTA.[crp-cysG]-10 mutation and transducing the linked traits into S. typhi Ty2 and the highly virulent S. typhi ISP1820 and ISP2822 strains via P22HTint-mediated transduction with selection for tetracycline resistance and screening for a maltose-negative phenotype. The zhc-1431::Tn10 linked to [crp-cysG]-10 and zid-62::Tn10 linked to cya were used for this purpose. Neither insertion alone affects virulence of S. typhimurium.

Detailed Description Text (138):
Genetic stability of avirulent mutants. Strains to be orally administered as live vaccines must have complete stability with regard to their avirulence attributes. When 50-fold concentrated cultures and various dilutions (.about.10.sup.9, 10.sup.7, 10.sup.5, 10.sup.3 CFU/plate) of the .DELTA.cya .DELTA.crp S. typhi strains were plated on minimal agar media (supplemented with required amino acids) containing 0.5% maltose, melibiose, xylose, glycerol, or rhamnose that should not support their growth, revertants and mutants were not detected. One set of duplicate plates was UV-irradiated (5 joules/meter.sup.2 /sec) and incubated at 37.degree. C. in the dark. The other set of plates was incubated at 37.degree. C. with illumination. Revertants and mutants were not detected after a 48 h growth period. An investigation was also conducted as to whether tetracycline-resistant revertants/mutants could be recovered at frequencies higher than could be observed for the parental strain. In all cases, such tetracycline-resistant revertants/mutants were not observed.

Detailed Description Text (140):
This Example describes the construction of recombinant avirulent S. typhi strains expressing foreign antigens for use as oral vaccines to immunize against various infectious diseases.

Detailed Description Text (144):
Construction of S. typhi strains with .DELTA.asdA1 mutation. The wild-type, virulent S. typhi Ty2 (type E1) was genetically modified as described below, using classical genetic methods similar to those described in Curtiss and Kelly (1987) and Nakayama, Kelly and Curtiss (1988). The construction of strains .chi.3927 and .chi.4323 containing the .DELTA.cya-12 .DELTA.crp-11 mutations was described in Example 5. The construction of strain .chi.4346 containing the .DELTA.[crp-cysG]-10 mutations was described in Example 6. The stable maintenance and high-level expression of cloned genes on recombinant plasmids in avirulent Salmonella strains is dependent upon use of a balanced-lethal host-vector system. For this, a chromosomal mutation of the asd gene encoding aspartate .beta.-semialdehyde dehydrogenase is introduced into a .DELTA.cya .DELTA.crp mutant to impose an obligate requirement for diaminopimelic acid (DAP) which is an essential constituent of the rigid layer of the bacterial cell wall and which is not synthesized in animals. The chromosomal .DELTA.asd mutation is then complemented by a plasmid cloning vector possessing the wild-type asd.sup.+ gene. Loss of the plasmid results in DAPless death and cell lysis. Such balanced-lethal host-vector combinations are stable for several weeks in the immunized animal host and elicit strong immune responses against the cloned gene

product as well as against Salmonella.

Detailed Description Text (153):
The S. typhi strains .chi.4297, .chi.4417 and .chi.4435 with the pYA1077 recombinant vector are candidates to immunize humans to protect against typhoid fever and leprosy. Efficacy of such vaccines will be dependent upon identifying one to several M. leprae antigens that would elicit protective immune responses and having them specified by cloned genes in an Asd.sup.+ vector in the S. typhi .DELTA.cya .DELTA.crp .DELTA.cdt .DELTA.asd strains which could then be used in human immunization trials.

Detailed Description Text (155):
This example provides a procedure for testing the safety, immunogenicity, and efficacy of live oral vaccines comprised of .DELTA.cya .DELTA.crp mutants of S. typhi. The strains tested are .DELTA.cya .DELTA.crp derivatives of Ty2, ISP1820 and ISP2822.

Detailed Description Text (174):
4. no history of any antibiotic therapy during the 7 days before vaccination;

Detailed Description Text (180):
Preparation of the vaccine inocula. Stock cultures of the S. typhi candidate vaccine strains are stored as a cell suspension in trypticase soy broth (TSB), supplemented with 15% glycerol, at -70.degree. C. until needed. To make an inoculum of each strain, the suspension is thawed and plated onto sheep red blood cell agar (5% srbc in TSA), two days before challenge. After incubation at 37.degree. C. overnight, about 20-30 typical colonies are picked and suspended in saline. This suspension is inoculated onto trypticase soy agar plates, appropriately supplemented, and the plates incubated overnight at 37.degree. C. In preparation for orally vaccinating the volunteers, growth on these plates is harvested with approximately 3 ml sterile normal saline per plate. The resulting suspension is standardized turbidimetrically. Dilutions are made in saline to approximate the concentration of Salmonella required. The vaccine inoculum is transported to the isolation ward on ice. Microscopic examination and slide agglutination with S. typhi O and H antisera are performed before use. Replica spread plate quantitative cultures are made of the inocula before and after vaccination to confirm viability and inoculum size.

Detailed Description Text (181):
Inoculation of Volunteers. The vaccine is administered by the oral route with NaHCO.sub.3. Volunteers are NPO for 90 minutes before vaccination. Two grams of NaHCO.sub.3 are dissolved in 5 ounces of distilled water. Volunteers drink 4 ounces of the bicarbonate water; one minute later the volunteers ingest the vaccine suspended in the remaining 1 ounce of bicarbonate water. Volunteers take no food or water for 90 minutes after inoculation.

Detailed Description Text (189):
Phlebotomy. Serum for antibody determinations is obtained before and 8, 21, 28, 60, and 180 days after vaccination. Heparinized blood for lymphocyte separations for antibody-secreting cell assays is collected on days 0, 4, 7, and 10. Mononuclear cells collected on days 0, 28, 60, and 180 days are used to assess lymphocyte proliferative responses to Salmonella and control antigens. Lastly mononuclear cells from days 0, 28, 60, and 180 are also used in the antibody-dependent cytotoxicity assay against S. typhi and control organisms. Blood (5 ml) is obtained for culture on days 3, 4, 7, 8, 10, 12, and 15 during the post-vaccination observation period to detect vaccine organisms. An additional specimen of serum and mononuclear cells are obtained 180 days after primary vaccination.

Detailed Description Text (190):
Jeiunal fluid aspiration. Before oral vaccination and immediately before discharge (day 15), volunteers

swallow polyvinyl chloride intestinal tubes to a distance of 130 cm from the mouth to collect intestinal fluid for measurement of local SIgA antibody. Ten mg of metoclopramide is given orally after ingestion of the tube to accelerate its passage from the stomach through the pylorus into the small intestine. Placement of the tubes in the jejunum is verified by distance (130 cm), color (yellow-green), and pH (6) of aspirated fluid. Approximately 100 ml of jejunal fluid is removed at each intubation.

Detailed Description Text (191):
Gelatin String Capsules. In order to determine rates of intestinal colonization with each vaccine strain, gelatin string capsules (Entero-Test) are ingested by volunteers three times during the period of hospitalization.

Detailed Description Text (193):
Tonsillar Cultures. In order to detect possible invasion of tonsillar lymph tissue after vaccination, serial tonsillar cultures are obtained on days 3, 4, 7, 8, 10, 12, and 15.

Detailed Description Text (194):
Bacteriological Analysis. Stools, rectal swabs, and the distal 15 cm of bile-stained duodenal string from the ingested gelatin capsule is inoculated into selenite F enrichment broth. Tonsillar swabs are inoculated into GN broth. After overnight incubation at 37.degree. C., subcultures are made onto Salmonella-Shigella agar and XLD agar, both appropriately supplemented for the auxotrophy of the vaccine strain. Suspicious colonies are transferred to supplemented triple sugar iron slants and confirmation made by agglutination with S. typhi Vi, O, and H antisera. These isolates are saved at -70.degree. C. in glycerol for further analysis (e.g., for the presence of plasmids or for Southern blotting with specific gene probes for cloned genes).

Detailed Description Text (196):
Immunological Analysis. Sera and jejunal fluid specimens are tested for IgA, IgM, and IgG antibodies to S. typhi O, H, and Vi antigens measured by ELISA, using the procedures described by Levine et al. (1987), J. Clin. Invest. 79:888-902. H antibody is also measured by Widal tube agglutination using S. virginia as antigen (S. virginia shares an identical flagellar antigen with S. typhi).

Detailed Description Text (198):
1. Antibody-secreting cells: trafficking lymphocytes which secrete IgG, IgA or IgM antibody against S. typhi O, Vi or H antigens are measured by the method of Kantele et al.

Detailed Description Text (201):
Excretion of the Vaccine Strain. It is expected that excretion of the vaccine strain would cease within 1 week after a dose of vaccine. If excretion continues for 7 or more days, the volunteer who continues to excrete is given a dose of ciprofloxacin (750 mg every 12 hours). Negative cultures for .gtoreq.2 consecutive days are required for discharge.

Detailed Description Text (204):
The procedure followed was essentially that described in Example 8, supra. Two cohorts of volunteers were used for studies in which different doses of vaccine were given. In the first study, 17 volunteers were randomized in a double-blind fashion; 6 volunteers received 5.times.10.sup.5 cfu of .chi.3927, the remainder received the same dose of other S. typhi strains. In the second study, 19 volunteers were randomized in a double-blind fashion; 6 volunteers received 5.times.10.sup.4 cfu of .chi.3927, the remainder received the same dose of other S. typhi strains. Volunteers were closely monitored on an Isolation Ward for 15 days (first study) or 24 days (second study). Vital signs were measured every six hours during the period of observation. All stools from each volunteer were collected in plastic containers, examined, graded on a five-point scale, and the volume measured if the stool was loose.

Volunteers were interviewed daily by a physician and asked about symptoms. Fever was defined as oral temperature .gtoreq.38.2.degree. C.; diarrhea was defined as two or more loose stools within 48 hours totalling at least 200 ml in volume or a single loose stool .gtoreq.300 ml in volume. Antibiotic therapy was given to volunteers who developed fever or positive blood cultures.

Detailed Description Text (205):
In order to prepare the vaccine, stock cultures of .chi.3927 which had been maintained on trypticase soy broth with 15% glycerol at -70.degree. C. were thawed and grown on supplemented aro agar. After incubation at 37.degree. C., 20-30 typical colonies of the vaccine strain were picked from aro agar, suspended in saline, and inoculated again onto aro agar. After overnight incubation at 37.degree. C., the bacteria were harvested with 3 ml of sterile phosphate buffered saline (PBS) and the concentration of bacteria was standardized turbidimetrically. Dilutions of the suspensions were made in PBS to achieve the desired concentration of viable organisms per milliliter. The identity of the inoculum was confirmed by microscopic examination and by side agglutination with S. typhi O, H, and Vi antisera. Replica spread plate quantitative cultures were made of the inocula before and after vaccination to confirm viability and the inoculum size.

Detailed Description Text (206):
The vaccine strains were administered by the oral route with sodium bicarbonate. Sodium bicarbonate (2 gm) was dissolved in 150 ml of distilled water and volunteers drank 120 ml to neutralize gastric acid. One minute later, volunteers drank the vaccine suspended in the remaining 30 ml of bicarbonate solution. Volunteers had nothing to eat or drink for 90 minutes before and after vaccination.

Detailed Description Text (207):
Every stool passed by volunteers (and rectal swabs if no stool was passed) was cultured daily for the vaccine strain. Stool was inoculated into Gram Negative broth (BBL, Cockeysville, Md.) supplemented with 0.1% PABA and 0.1% PHB and directly onto S--S agar with supplements. After incubation overnight at 37.degree. C., subcultures were made onto supplemented S--S agar. To quantitate the shedding of vaccine strains, 1 g of stool was serially diluted 10-fold in saline and each dilution was plated onto S--S agar supplemented as above. Suspicious colonies were transferred to triple sugar iron agar slants and the identity confirmed by agglutination with S. typhi O, H, and Vi antisera.

Detailed Description Text (208):
On days 7, 10, and 13 after vaccination, fasting volunteers swallowed gelatin capsules containing string devices to collect samples of bile-stained duodenal fluid. After 4 hours, the strings were removed and the color and pH of the distal 15 cm were recorded. Duodenal fluid was squeezed from the end of the string and cultured as above.

Detailed Description Text (209):
Blood for culture of the vaccine organisms was systematically collected on days 4, 5, 7, 8, 10, 12, and 15 after vaccination and again if fever occurred. Five ml of blood was inoculated into 50 ml of supplemented aro broth.

Detailed Description Text (210):
In addition, tonsillar cultures were obtained on days 1, 2, 4, 5, 7, 8, 10, 12 and 15 to detect the vaccine strain. Swabs applied to the tonsils were inoculated into Gram Negative broth with supplements for 24 hours and then onto supplemented salmonella-shigella agar.

Detailed Description Text (211):
In order to determine the immunological response, the following procedures were followed. Serum samples were obtained before and on days 7, 21, 28, and 60 after vaccination. Jejunal fluids were

collected before and on day 14 after vaccination, as described in Example 8. The total IgA content of the fluids were measured by ELISA and each specimen was standardized to contain 20 mg of IgA per 100. Antibodies to S. typhi lipopolysaccharide (LPS), H, and Vi antigens were measured in serum and jejunal fluids.

Detailed Description Text (212):
IgG antibody to LPS O antigen was detected by ELISA. A rise in net optical density .gtoreq.0.20 between pre- and post-vaccination sera tested at a 1:100 dilution was considered a significant rise. The positive control serum used with each microtiter plate contained a high level of LPS O antibody and represented a pool of sera from 12 healthy Chileans who had strong IgG LPS O antibody responses after immunization with Ty21a vaccine. IgA antibody to LPS O antigen was measured using two-fold dilutions of serum, starting with a 1:25 dilution. An IgA titer was considered significant if a 4-fold rise occurred between pre- and post-vaccination procedures.

Detailed Description Text (213):
Intestinal secretory IgA antibody to S. typhi LPS O antigen was also measured by ELISA. Four-fold rises were considered significant.

Detailed Description Text (217):
Gut-derived, trafficking antibody secreting cells (ASC) that secrete IgG, IgA, or IgM antibody against S. typhi O, H, or Vi antigens were measured by a modification of the method of Forrest et al. ((1988), Lancet 1:81) using both ELISA and ELISPOT assays. Heparinized blood was drawn before and on days 7 and 10 after vaccination. Briefly, peripheral blood lymphocytes separated by a Ficoll gradient (Organon Teknika, Durham, N.C.) were added to antigen-coated plates. In the ELISA, binding of antibody secreted by lymphocytes was measured by the change in optical density produced by the reaction of the substrate with bound anti-IgA conjugate. Significant responses to LPS, H, and Vi antigens were determined using the differences in O.D. pllus 3 S.D. generated from pre-immunization and day 4 cells taken from volunteers participating in these studies. In the ELISPOT assay, specific IgA secreted by individual lymphocytes was detected by adding an agarose overlay to each well and counting colored spots produced by reaction of the substrate with bound anti-human IgA conjugate. Detection of .gtoreq.4 spots per well after vaccination was defined as a positive response; this number is based on the mean number of spots counted before vaccination plus 2 S.D.

Detailed Description Text (219):
The clinical signs and symptoms of volunteers after vaccination were evaluated in a double-blind fashion. One of 12 volunteers who received strain .chi.3927 had fever. This volunteer developed fever with a maximum temperature of 40.1.degree. C. on day 22 after vaccination. This volunteer had severe abdominal cramps, malaise, anorexia, headache, and vomiting on days 4-13, but his fever did not begin until day 22. His symptoms then included dizziness, muscle and body aches, constipation, insomnia, and cough productive of brown sputum. Another volunteer in this group had malaise, cramps, headache, and nausea during the inpatient surveillance period.

Detailed Description Text (220):
The bacteriology studies showed that one of six volunteers who received 5.times.10.sup.4 and one of six volunteers who received 5.times.10.sup.5 cfu of .chi.3927 had positive blood cultures. These occurred on days 15 and days 8 and 12, respectively. Neither of these volunteers had any symptoms. One of the 12 volunteers who received .chi.3927 had one colony of vaccine organisms detected in the stool on day 1. None of these volunteers had positive tonsillar or duodenal string cultures. The .chi.3927 isolates recovered from the blood and the stool of volunteers retained all expected phenotypes associated with the presence of .DELTA.cya .DELTA.crp mutations.

Detailed Description Text (221):
The immunological studies showed that six (50%) of the 12 vaccines who received .chi.3927 developed IgG anti-S. typhi LPS responses. No antibody to H antigen or Vi were detected in any of the twelve volunteers. Only one of the twelve volunteers developed secretory IgA against LPS in the jejunal fluid. Secretory IgA antibody responses to H antigen occurred in only one volunteer and no volunteer had secretory anti-Vi antibody after vaccination. Five of 12 volunteers developed circulating cells secreting IgA against LPS detected by ELISA or ELISASPOT assay.

Detailed Description Text (222):
The degree of attenuation conferred by deletions in the cyclic AMP regulatory pathway cannot be strictly measured without simultaneous challenge of volunteers with mutant and parent strains. However, based on historical experience with volunteers given similar doses of wild type strains, it is likely that the deletions confer attenuation to S. typhi. When wild-type S. typhi strain Ty2 was fed to six volunteers at a dose of 1.times.10.sup.7 without bicarbonate, 83% developed typhoid fever (defined as temperature 103.degree. F. for >36 hours) or infection (defined as low grade fever, significant serologic response, positive blood culture, or excretion of S. typhi for >5 days. In contrast, among the 12 volunteers reported herein who received the .chi.3927 vaccine derived from Ty2 at a dose of 10.sup.4 or 10.sup.5 cfu with bicarbonate (equivalent to a much higher dose without bicarbonate), fever occurred in only one volunteer and positive blood cultures in only two volunteers. Moreover, volunteers who had febrile illnesses did not have vaccine bacteria detected in their blood, despite additional blood cultures collected at the time of fever. It is likely that fever occurred in response to the release of cytokines stimulated by the enteric infection with the vaccine.

Detailed Description Text (241):
The following is a standardized protocol for growth and suspension of each vaccine strain and its parent for intraperitoneal (i.p.) inoculation of mice.

Detailed Description Text (251):
The strains provided herein are directly and indirectly suitable for the production of immunogenic compositions, including vaccines, to prevent diseases caused by S. typhi, and other enteric bacteria with which antibodies to S. typhi cross react. These strains are also useful as carrier microorganisms for the production of expression products encoded on recombinant genes in the bacterial cells. In addition, the strains which can be used with enhanced safety are useful for the production of antibodies, both monoclonal and polyclonal, against S. typhi, and against antigens which are expressed in the avirulent S. typhi.

Detailed Description Paragraph Footnote (1):
.sup.1 Luria broth contains 10 g of NaCl per liter whereas Lennox broth contains 5 g of NaCl per liter. It has been shown that Salmonella cells grown in high osmolarity media display an increased ability to invade tissue culture cells (Galan and Curtiss, Infect. Immun. (1990) 58:1879-1885; expression of Salmonella genes required for invasion is regulated by changes in DNA supercoiling). Therefore, the increased NaCl level in Luria broth ensures optimal effectiveness of the vaccine strain.

Detailed Description Paragraph Table (2):
.chi.3957 pSD110.sup.+ .DELTA.[crp-cysG]-10 Fusaric acid-resistant, tetracycline- .DELTA.[zhc-1431::Tn10] .DELTA.cya-12 sensitive, Mal.sup.-, Cys.sup.-, Arg.sup.- derivativ e of .DELTA.[zid-61::Tn10] .chi.3956 .chi.3958 .DELTA.[crp-cysG]-10 .DELTA.[zhc-1431::Tn10] Ampicillin-sensitive derivative of .chi.3957; .DELTA.cya-12 .DELTA.[zid-61::Tn10] pSD110 cured by serial passage in L broth at 37.degree. C. .chi.3961 pSD110.sup.+ .DELTA.crp-11 .DELTA.[zhc-1431::Tn10] P22HTint (.chi.3670) .chi.3954 with selection for ampicillin resistance, Mal.sup.+. .chi.3962 pSD110.sup.+ .DELTA.crp-11 .DELTA.[zhc-1431::Tn10] P22HTint(.chi.3711) .chi.3961 with selection

for .DELTA.cya-12 zid-62::Tn10 tetracycline resistance, Mal.sup.-. .chi.3978 pSD110.sup.+ .DELTA.crp-11 .DELTA.[zhc-1431::Tn10] P22HTint(.chi.3711) .chi.3938 with selection for .DELTA.cya-12 zid-62::Tn10 tetracycline resistance, Mal.sup.-. .chi.3985 .DELTA.cya-12 .DELTA.[zid-62::Tn10] ATCC68166; Fusaric acid-resistant .DELTA.crp-11 .DELTA.[zhc-1431::Tn10] tetracycline-sensitive, Mal.sup.- derivative of .chi.3962 cured of pSD110. .chi.4038 .DELTA.cya-12 .DELTA.[zid-62::Tn10] Fusaric acid-resistant tetracycline- .DELTA.[crp-cysG]-10 .DELTA.[zhc-1431::Tn10] sensitive Mal.sup.-, Cys.sup.-, Arg.sup.- derivative of .chi.3902 cured of pSD110. .chi.4039 .DELTA.cya-12 .DELTA.[zid-62::Tn10] Fusaric acid-resistant, tetracycline- .DELTA.[crp-cysG]-10 .DELTA.[zhc-1431::Tn10] sensitive Mal.sup.- derivative of .chi.3978 cured of pSD110. .chi.4063 SR-11 arg::Tn10 P22HTint(Tn10 library) .chi.3306 with selection for tetracycline resistance, Arg.sup.-. .chi.4071 SR-11 arg::Tn10 P22HTint(Tn10 library) .chi.3306 with selection for tetracycline resistance, Arg.sup.-. .chi.4246 .DELTA.[crp-cysG]-10 zhc-1431::Tn10. P22HTint(.chi.3712) 798 with selection for tetracycline resistance, Mal.sup.-, (Cys.sup.- Arg.sup.-). .chi.4247 pSD110.sup.+ .DELTA.[crp-cysG]-10 P22HTint(.chi.3670) .chi.4246 with selection for zhc-1431::Tn10 ampicillin resistance, Mal.sup.+, (Cys.sup.- Arg.sup.-). .chi.4248 .DELTA.[crp-cysG]-10 zhc-1431::Tn10 P22HTint (.chi.3712) ATCC68169 (UK-1) with selection for tetracycline resistance, Mal.sup.-, (Cys.sup.- Arg.sup.-). .chi.4262 pSD110.sup.+ [crp-cysG]-10 P22HTint(.chi.3670) .chi.4248 with selection for zhc-1431::Tn10 ampicillin resistance, Mal.sup.+, (Cys.sup.- Arg.sup.-). C. S. typhi Ty2 Type E1 Cys.sup.- Trp.sup.- wild type Louis Baron, Walter Reed Army Institute of Research. ISP1820 Type 46 Cys.sup.- Trp.sup.- wild type Center for Vaccine Development, Baltimore, MD; 1983 isolate for Chilean patient. ISP2822 Type E1 Cys.sup.- Trp.sup.- wild type Center for Vaccine Development, Baltimore, MD; 1983 isolate for Chilean patient. .chi.3791 .DELTA.[crp-cysG]-10 zhc-1431::Tn10 P22HTint (.chi.3712) ISP2822 with selection for tetracycline resistance (Mal.sup.-, Cys.sup.-, Arg.sup.-, Vi.sup.+). .chi.3792 .DELTA.[crp-cysG]-10 zhc-1431::Tn10 P22HTint(.chi.3712) Ty2 with selection for tetracycline resistance (Mal.sup.-, Cys.sup.-, Arg.sup.- Vi.sup.+). .chi.3802 .DELTA.[crp-cysG]-10 .DELTA.[zhc-1431::Tn10] Fusaric acid-resistant, tetracycline- sensitive Mal.sup.- derivative of .chi.3791 (Vi.sup.+). .chi.3803 .DELTA.[crp-cysG]-10 .DELTA.[zhc-1431::Tn10] Fusaric acid-resistant, tetracycline- sensitive Mal.sup.- derivative of .chi.3792 (Vi.sup.+). .chi.3824 pSD110.sup.+ .DELTA.[crp-cysG]-10 .chi.3803 electro-transformed with pSD110 from .DELTA.[zhc-1431::Tn10] .chi.3670 with selection for ampicillin resistance (Mal.sup.+, Cys.sup.-, Arg.sup.-, Vi.sup.+). .chi.3845 pSD110.sup.+ .DELTA.[crp-cysG]-10 .chi.3802 electro-transformed with pSD110 form .DELTA.[zhc-1431::Tn10] .chi.3670 with selection for ampicillin resistance (Mal.sup.+, Cys.sup.-, Arg.sup.-, Vi.sup.+). .chi.3852 .DELTA.crp-11 zhc-1431::Tn10 P22HTint(.DELTA.3773) ISP2822 with selection for tetracycline resistance (Mal.sup.-, Vi+). .chi.3853 .DELTA.crp-11 zhc-1431::Tn10 P22HTint(.chi.3773) Ty2 with selection for tetracycline resistance (Mal.sup.-, Vi.sup.+). .chi.3877 .DELTA.crp-11 .DELTA.[zhc-1431::Tn10] Fusaric acid-resistant, tetracycline- sensitive Mal.sup.- derivative of .chi.3852 (Vi.sup.+). .chi.3878 .DELTA.crp-11 .DELTA.[zhc-1431::Tn10] Fusaric acid-resistant, tetracycline- sensitive Mal.sup.- derivative of .chi.3853 (Vi.sup.+). .chi.3879 pSD110.sup.+ .DELTA.crp-11 .DELTA.[zhc-1431::Tn10] P22HTint(.chi.3670) .DELTA.3877 with selection for ampicillin resistance (Mal.sup.+, Vi.sup.+). .chi.3880 pSD110.sup.+ .DELTA.crp-11 .DELTA.[zhc-1431::Tn10] P22HTint(.chi.3670) .chi.3878 with selection for ampicillin resistance (Mal.sup.+, Vi.sup.+). .chi.3919 pSD110.sup.+ [crp-cysG]-10 P22HTint(.chi.3711) .lambda. .chi.3824 with selection for .DELTA.[zhc-1431::Tn10] .DELTA.cya-12 tetracycline (Mal.sup.-, Vi.sup.+). zid-62::Tn10 .chi.3920 pSD110.sup.+ .DELTA.[crp-cysG]-10 P22HTint(.chi.3711) .chi.3845 with selection for .DELTA.[zhc-1431::Tn10] .DELTA.cya-12 zid-62::Tn10 .chi.3921 pSD110.sup.+ .DELTA.crp-11 .DELTA.[zhc-1431::Tn10] P22HTint(.chi.3711) .chi.3879 with selection .DELTA.cya-12 zid-62::Tn10 for tetracycline resistance (Mal.sup.-, Vi.sup.+). .chi.3922 pSD110.sup.+ .DELTA.crp-11 .DELTA.[zhc-1431::Tn10] P22HTint(.chi.3711) .chi.3880 with selection .DELTA.cya-12 zid-62::Tn10 for tetracycline resistance (Mal-, Vi.sup.+). .chi.3924 .DELTA.[crp-cysG]-10 .DELTA.[zhc-1431::Tn10] Fusaric acid-resistant, tetracycline- .DELTA.cya-12 .DELTA.[zid-62::Tn10] sensitive Mal.sup.- derivative of .chi.3919 cured of pSD110 (Vi.sup.+). .chi.3925 .DELTA.[crp-cysG]-

10 .DELTA.[zhc-1431::Tn10] Fusaric acid-resistant, tetracycline- .DELTA.cya-12 .DELTA.[zid-62::Tn10] sensitive Mal.sup.- derivative of .chi.3920 cured of pSD110 (Vi.sup.+). .chi.3926 .DELTA.crp-11 .DELTA.[zhc-1431::Tn10] Fusaric acid-resistant, tetracycline- .DELTA.cya-12 .DELTA.[zid-62::Tn10] sensitive Mal.sup.- derivative of .chi.3921 cured of pSD110 (Vi.sup.+). .chi.3927 .DELTA.crp-11 .DELTA.[zhc-1431::Tn10] Fusaric acid-resistant, tetracycline- .DELTA.cya-12 .DELTA.[zid-62::Tn10] sensitive Mal.sup.- derivative of .chi.3922 cured of pSD110 (Vi.sup.+). .chi.3940 .DELTA.[crp-cysG]-10 .DELTA.[zhc-1431::Tn10] Flagella-positive, motile derivative of .DELTA.cya-12 .DELTA.[zid-62::Tn10] .chi.3925 (Vi.sup.+). .chi.4073 .DELTA.[crp-cysG]-10 .DELTA.[zhc-1431::Tn10] Flagella-positive, motile derivative of .DELTA.cya-12 .DELTA.[zid-62::Tn10] .chi.3924 (Vi.sup.+). .chi.4296 .DELTA.crp-11 .DELTA.[zhc-1431::Tn10] P22HTint (.chi.3520) .chi.3927 with selection .DELTA.cya-12 .DELTA.[zid-62::Tn10] for tetracycline resistance and screening .DELTA.asdA1 zhf-4::Tn10 for Asd.sup.-, Mal.sup.-, Vi.sup.+. .chi.4297 .DELTA.crp-11 .DELTA.[zhc-1431::Tn10] Fusaric acid-resistant, tetracycline- .DELTA.cya-12 .DELTA.[zid-62::Tn10] sensitive Asd.sup.-, Mal.sup.- derivative of .chi.4296 .DELTA.asdA1 .DELTA.[zhf-4::Tn10] (Vi.sup.+). .chi.4298 .DELTA.crp-11 zhc-1431::Tn10 P22HTint(.chi.3773) ISP1820 with selection for tetracycline resistance (Mal.sup.-, Vi.sup.+). .chi.4299 .DELTA.crp-11 .DELTA.[zhc-1431::Tn10] Fusaric acid-resistant, tetracycline- sensitive Mal.sup.- derivative of .chi.4298 (Vi.sup.+). .chi.4300 pSD110.sup.+ .DELTA.crp-11 .DELTA.[zhc-1431::Tn10] P22HTint(.chi.3670 .DELTA.4299 with selection for ampicillin resistance (Mal.sup.+, Vi.sup.+). .chi.4316 pSD110.sup.+ .DELTA.crp-11 .DELTA.[zhc-1431::Tn10] P22HTint(.chi.3670) .chi.4300 with .DELTA.cya-12 zid-62::Tn10 selection for tetracycline resistance (Mal.sup.-, Vi.sup.+). .chi.4322 .DELTA.crp-11 .DELTA.[zhc-1431::Tn10] Fusaric acid-resistant, tetracycline- .DELTA.cya-12 .DELTA.[zid-62::Tn10] sensitive Mal.sup.- derivative of .chi.4316 cured pSD110 (Vi.sup.+). .chi.4323 .DELTA.crp-11 .DELTA.[zhc-1431::Tn10] Flagella-positive, motile derivative .DELTA.cya-12 .DELTA.[zid-62::Tn10] of .chi.4322 (Vi.sup.+) .chi.4324 .DELTA.[crp-cysG]-10 zhc-1431::Tn10

Detailed Description Paragraph Table (12):

TABLE 10 _____ Bacterial Strains

_____ .chi.3769, S. typhi Ty2 Type E1, wild type, Vi.sup.+. Received from L. Baron, Walter Reed Army Institute of Research, Washington, DC, as Ty2. .chi.4073 S. typhi Ty2 .DELTA.crp-10 [zhc-1431::Tn10] .DELTA.cya-12 .DELTA.[zid- 62::Tn10]; Crp.sup.- Cdt.sup.- Cya.sup.- Arg.sup.- derivative of .chi.3769. .chi.3744 S. typhi ISP1820 Type 46, wild type, Vi.sup.+. Received from M. Levine, Center for Vaccine Development, Baltimore, MD, as ISP1820. 1983 isolate from a Chilean patient. .chi.4346 S. typhi ISO1820 .DELTA.crp-10 .DELTA.[zhc-1431::Tn10] .DELTA.cya-12 .DELTA.[zid- 62::Tn10]; Crp.sup.- Cd.sup.- Cya.sup.- Arg.sup.- derivative of .chi.3744. _____

CLAIMS:

2. An immunogenic composition for the immunization of an individual according to claim 1 wherein the avirulent derivative of a pathogenic gram negative bacteria is capable of expressing a recombinant gene derived from an agent which is pathogenic to said individual, to produce an antigen capable of inducing an immune response in said vertebrate against said pathogenic agent.

3. A method for stimulating the immune system to respond to an immunogenic antigen of a pathogenic gram negative bacteria comprising administering to said individual the immunogenic composition of claim 1.

4. A method for stimulating the immune system to respond to an immunogenic antigen of a pathogen comprising administering to said individual the immunogenic composition of claim 2.

6. The isolated bacterial strain of claim 5 which is capable of expressing a recombinant gene derived from an agent which is pathogenic to an individual, to produce an antigen capable of inducing an immune response in said individual against said pathogenic agent.

7. A strain according to claim 6, wherein the avirulent strain of a pathogenic gram negative bacteria contains a chromosomal mutation which is lethal, balanced by a vector gene which complements the lethal mutation to constitute a balanced-lethal host-vector system.

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L5: Entry 235 of 264 File: USPT Oct 28, 1997

DOCUMENT-IDENTIFIER: US 5681745 A
** See image for Certificate of Correction **
TITLE: Biotin-binding containment systems

Detailed Description Text (24):
A streptavidin-based containment system can be used to control the survival of engineered microorganisms genetically constructed to degrade hazardous chemicals such as hydrocarbons, aromatic compounds and halogenated compounds including toluates, xylenes, benzenes, polycyclic hydrocarbons and derivatives and combinations of these compounds. According to the invention, microorganisms can be genetically altered to couple the control of degradation of different types of hazardous organic chemicals to the survival of the organism. This control is manifested in the self-destruction of the microorganism once the target chemicals in the environment have been degraded. This type of genetic control will function in laboratory or controlled fermentation settings, or in the field in actual bioremediation applications. The control of survival is based on expression of streptavidin protein which is only produced once the target chemical is degraded. Once there is no further target chemical present, or a critical lower threshold is reached, the gene encoding the streptavidin protein is expressed, and the organism is killed due to complexation of all the essential biotin vitamin in the cell with the newly expressed streptavidin. Aside from controlled fermentations and field remediation needs, this same containment or control system may be useful for the delivery of live vaccines and biopesticides, or a range of bioengineered products both in the field or in controlled laboratory environments.

Detailed Description Text (26):
Streptavidin-based genetic systems are also useful for environmental remediation. Both biological and non-biological treatments for contaminated soils and waters can be performed and at a lower effective cost. This same technology can also be used in biopesticide delivery or vaccine delivery as a way to control the organisms used in some of these processes. In addition, many suicide functions are being developed to control genetic elements such as vital and retroviral vectors, during gene therapy for medical treatments. The system described herein is adaptable for these applications as well. This technology can also be used to control cell lines involved in the production of important pharmaceuticals or drugs. It is often critical to control organisms in fermentation systems to assure that all organisms are killed after their useful life cycle in the production process. Should viable cells get out, carefully selected strains could be lost. Thus, the genetic containment system described may also be useful as a security system to provide enhanced assurance that there is no chance of release of viable organisms once growth is completed on a particular substrate.

Detailed Description Text (38):
With this design, lethal expression of the stv gene is tightly controlled by the bacteriophage T7 transcription system, that is the .o slashed.10 promoter, the RNA polymerase encoded by the T7 gene 1 fused with Escherichia coli P.sub.tac promoter, and the lysozyme, here as an inhibitor of RNA polymerase. This entire containment system can be conditioned by the E. coli lacI repressor gene fused with a promoter responding to environmental or physiological changes. A plasmid-based construct was examined in E. coli and Pseudomonas putida. Induction of stv gene expression resulted in cell-killing with efficiency up to 99.9%. Mutants escaping killing

appeared at frequencies reaching 10.sup.-6 -10.sup.-7 per cell per generation. The general requirement for biotin in the living systems makes the stv cassette a candidate for containment strategies in a broad range of microorganisms.

Entry 235 of 264 File: USPT Oct 28, 1997

US-PAT-NO: 5681745
DOCUMENT-IDENTIFIER: US 5681745 A
** See image for Certificate of Correction **

TITLE: Biotin-binding containment systems

DATE-ISSUED: October 28, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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Sano; Takeshi	Boston	MA		
Marx; Kenneth A.	Francestown	NH		
Cantor; Charles R.	Boston	MA		
Kaplan; David L.	Stow	MA		
Smith; Cassandra L.	Boston	MA		

US-CL-CURRENT: 435/325; 435/252.31, 435/252.33, 435/257.2, 435/320.1, 435/410, 536/23.7, 536/24.1

CLAIMS:

We claim:

1. A genetic comment system comprising a cassette that encodes a biotin-binding binding component.

2. The containment system of claim 1 wherein the biotin-binding component is a protein selected from the group consisting of streptavidin, avidin and mutants thereof.

3. The containment system of claim 2 wherein the streptavidin mutation is a core streptavidin protein.

4. The genetic containment system of claim 1 wherein the cassette comprises a regulatable promoter and a suicide gene.

5. The containment system of claim 4 wherein the regulatable promoter is functionally linked to said suicide gene.

6. The containment system of claim 4 wherein the suicide gene is selected from the group consisting of E. coli gef genes, Serratia marcescens and Staphylococcus aureus endonucleases, Serratia liquefaciens phospholipase A, Bacillus subtilis sacB gene, lysis genes from bacteriophages and combinations thereof.

7. The containment system of claim 4 wherein the suicide gene encodes said

biotin-binding component.

8. The containment system of claim 4 wherein the regulatable promoter is selected from the group consisting of bacteriophage SP6 promoters, bacteriophage T7 promoters, bacteriophage T3 promoters, bacteriophage .gamma. p.sub.L promoters, trp promoters, lac promoters, hybrid trp-lac promoters, phoA phoA promoters, gal promoters, metallothionein promoters, MMTV promoters and hybrids and combinations thereof.

9. The containment system of claim 4 wherein the regulatable promoter is an inducible promoter selected from the group consisting of phage inducible promoters, nutrient inducible promoters, temperature inducible promoter, radiation inducible promoters, metal inducible promoters, hormone inducible promoters, steroid inducible promoters and hybrids and combinations thereof.

10. The containment system of claim 4 wherein the regulatable promoter is regulated by a transcriptional effector.

11. The containment system of claim 10 wherein the transcriptional effector is a transcriptional repressor or a transcriptional activator.

12. The containment system of claim 10 wherein the transcriptional effector is an RNA polymerase.

13. The containment system of claim 10 wherein the regulatable promoter is a bacteriophage T7 promoter and the transcriptional effector is a T7 RNA polymerase.

14. The containment system of claim 10 wherein the transcriptional effector is encoded within said genetic containment system.

15. The containment system of claim 10 wherein the regulatable promoter is functionally linked to a genetic element that is regulated by said transcriptional effector.

16. The containment system of claim 10 wherein the regulatable promoter is induced by one or more physiological conditions.

17. The containment system of claim 16 wherein the physiological conditions are are selected from the group consisting of changes in pH, temperature, radiation, osmotic pressure, saline gradients, cell surface binding and the concentration of one or more extrinsic or intrinsic agents.

18. The containment system of claim 17 wherein the extrinsic agent is selected from the group consisting of amino acids and amino acid analogs, saccharides and polysaccharides, nucleic acids, transcriptional activators and repressors, cytokines, toxins, petroleum-based compounds, metal containing compounds, salts, ions, enzyme substrate analogs and combinations thereof.

19. The containment system of claim 10 further comprising an inhibitor of the transcriptional effector.

20. The containment system of claim 19 wherein the inhibitor is encoded within said genetic containment system.

21. The containment system of claim 19 wherein the inhibitor is constituitively

constituitively expressed.

22. The containment system of claim 19 wherein the inhibitor is a substrate analog.

23. The containment system of claim 19 wherein the transcriptional effector is a T7 RNA polymerase and the inhibitor is a T7 lysozyme.

24. A genetic containment system comprised of a cassette that encodes a suicide suicide gene and a marker gene that encodes a biotin-binding protein.

25. The containment system of claim 24 wherein the marker gene encodes streptavidin protein, avidin protein or mutants thereof.

26. A vector that contains a genetic containment system comprised of a suicide gene that encodes a biotin-binding protein whose expression is regulated by a transcriptional effector encoded within said vector wherein expression of said transcriptional effector is regulated by a physiological condition.

27. The nucleic acid of claim 26 wherein the genetic vector is a plasmid, viral viral vector, cosmid, phage vector or combination thereof.

28. The nucleic acid of claim 26 wherein the streptavidin gene is functionally linked to a regulatable promoter.

29. The nucleic acid of claim 28 wherein the regulatable promoter is selected from the group consisting of bacteriophage SP6 promoters, bacteriophage T7 promoters, bacteriophage T3 promoters, bacteriophage .gamma. p.sub.L promoters, promoters, trp promoters, lac promoters, hybrid trp-lac promoters, phoA promoters, gal promoters, metallothionein promoters, MMTV promoters and combinations thereof.

30. The nucleic acid of claim 28 wherein the regulatable promoter is an inducible promoter selected from the group consisting of phage inducible promoters, nutrient inducible promoters, temperature inducible promoter, metal inducible promoters, hormone inducible promoters, steroid inducible promoters and combinations thereof.

31. The nucleic acid of claim 26 wherein the physiological condition is selected from the group consisting of a changes in pH, temperature, radiation, osmotic pressure, saline gradients and concentration of an extrinsic or intrinsic agent.

32. The nucleic acid of claim 31 wherein the extrinsic or intrinsic agent is selected from the group consisting of amino acids and amino acid analogs, saccharides and polysaccharides, nucleic acids, transcriptional activators and repressors, cytokines, toxins, petroleum-based compounds, metal containing compounds, salts, ions, enzyme substrate analogs and combinations thereof.

33. The nucleic acid of claim 31 wherein the extrinsic agent is IPTG or an analog thereof.

34. The nucleic acid of claim 26 further comprising an inhibitor of the transcriptional effector.

35. The nucleic acid of claim 34 wherein the inhibitor is constituitively

expressed.

36. The nucleic acid of claim 34 wherein the transcriptional effector is T7 RNA RNA polymerase and the inhibitor is T7 lysozyme.

37. A recombinant cell containing a genetic containment system comprised of a suicide cassette that encodes a biotin-binding protein.

38. The cell of claim 37 which is a prokaryotic or a eukaryotic cell.

39. The cell of claim 38 wherein the eukaryotic cell is a plant cell, an algae cell or a mammalian cell.

40. The cell of claim 38 wherein the prokaryotic cell is a gram-negative or a gram-positive bacterial cell.

41. A genetic containment system comprising a suicide gene encoding a biotin-binding protein functionally linked to an inducible promoter, a suicide control control gene encoding a heterologous polymerase functionally linked to a repressible promoter regulated by a repressor, and a polymerase control gene functionally linked to a constitutive promoter.

42. The genetic containment system of claim 41 wherein the suicide gene encodes encodes streptavidin, avidin or mutants.

43. The genetic containment system of claim 41 wherein the inducible promoter is a bacteriophage .gamma., SP6, T3 or T7 promoter.

44. The genetic containment system of claim 41 wherein the heterologous polymerase is a bacteriophage, SP6, T3 or T7 polymerase.

45. The genetic containment system of claim 41 wherein the repressor is a lactose, galactose or tryptophan repressor protein.

46. The genetic containment system of claim 41 wherein the repressible promoter promoter is P.sub.tac, P.sub.lac, P.sub.trp, P.sub.gal, P.sub.phoA or a hybrid thereof.

47. The genetic containment system of claim 41 wherein the polymerase control gene encodes a lysozyme.

48. The genetic containment system of claim 41 wherein the constitutive promoter is P.sub.m.

49. The genetic containment system of claim 41 further comprising a gene encoding the repressor functionally linked to another inducible promoter.

50. The genetic containment system of claim 49 wherein the another inducible promoter that is activated or repressed in response to a change of an environmental condition.

51. The genetic containment system of claim 50 wherein the environmental condition is the change in concentration of a chemical, metal, radiation or nutrient or change in pH.

52. A genetic containment system comprising a suicide gene encoding a biotin-binding protein functionally linked to an inducible promoter, a suicide control control gene encoding a polymerase functionally linked to a repressible promoter, a polymerase control gene functionally linked to a constitutive promoter, and a gene that encodes said repressor functionally linked to another another inducible promoter that responds to an environmental condition.

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L5: Entry 132 of 264 File: USPT Aug 24, 2004

US-PAT-NO: 6780405
DOCUMENT-IDENTIFIER: US 6780405 B1

TITLE: Regulated antigen delivery system (RADS)

DATE-ISSUED: August 24, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Curtiss, III; Roy	St. Louis	MO		
Tinge; Steven A.	Belleville	IL		

US-CL-CURRENT: 424/93.1; 424/200.1, 424/93.2, 424/93.4, 435/252.3, 435/320.1

CLAIMS:

What is claimed is:

1. A microorganism comprising a regulated antigen delivery system (RADS), wherein the RADS comprises (a) a vector comprising (1) a gene encoding a desired gene product inserted into a site for insertion of a gene encoding a desired gene product, wherein the gene encoding the desired gene product is operably linked to a second control sequence; (2) a first origin of replication replication (ori) conferring vector replication using DNA polymerase III; and (3) a second ori conferring vector replication using DNA polymerase I, wherein the second ori is operably linked to a first control sequence repressible by a first repressor, and wherein the runaway vector does not comprise a phage lysis lysis gene; and (b) a gene encoding a first repressor operably linked to a first activatible control sequence.

2. The microorganism of claim 1, wherein the first control sequence and the second control sequence are the same sequence.

3. The microorganism of claim 1, wherein the first control sequence and the second control sequence are different sequences.

4. The microorganism of claim 1, wherein the repressor is selected from the group consisting of LacI repressor and C2 repressor, and wherein the second control sequence is repressible by a second repressor.

5. The microorganism of claim 1, wherein (a) the vector is a plasmid; (b) the desired gene product is an antigen and (c) the microorganism is an attenuated bacterium.

6. The microorganism of claim 5, wherein the microorganism is a Salmonella sp.

7. The microorganism of claim 5, wherein the first activatible control sequence sequence is araCP.sub.BAD.

8. The microorganism of claim 5, further comprising an inactivating mutation in in a native gene selected from the group consisting of cya, crp, phoPQ, opmR, galE, cdt, hemA, aroA, aroC, aroD and htrA.

9. The microorganism of claim 5, wherein the first ori is a pSC ori, and the second ori is a pUC ori.

10. The microorganism of claim 5, wherein the first control sequence is P22 P.sub.R and the first repressor is C2 repressor.

11. The microorganism of claim 5, wherein the second control sequence is P.sub.trc and wherein the second control sequence is repressible by a second repressor, and wherein the second repressor is a LacI repressor.

12. The microorganism of claim 11, wherein the first control sequence is P22 P.sub.R : the first repressor is C2 repressor; the first ori is a pSC ori, and the second ori is a pUC ori.

13. The microorganism of claim 12, wherein the vector is pMEG-771with a gene encoding an antigen.

14. The microorganism of claim 5, wherein the antigen is selected from the group consisting of Ery65 and SeM.

15. The microorganism of claim 5, wherein the desired gene product is operably linked to a eukaryotic control sequence.

16. The microorganism of claim 15, further comprising a .DELTA.endA mutation.

17. A runaway vector comprising the vector in the microorganism of claim 15.

18. The microorganism of claim 5, which exhibits delayed RADS characteristics, wherein the delayed RADS characteristics are conferred by an aeration selected from the group consisting of: mutations that delay the loss of activator molecules by metabolism and/or leakage, a mutation or insertion to increase repressor concentration, and inclusion of a vector control sequence with binding sites for more than one repressor and/or vector sequences encoding repressor molecules that act on a vector control sequence.

19. A vaccine for immunization of a vertebrate, the vaccine comprising the microorganism of claim 5, in a pharmaceutically acceptable carrier.

20. The vaccine of claim 19, wherein the microorganism is a Salmonella sp.

21. The vaccine of claim 19, wherein: (a) the first ori is a pSC orl; (b) the second ori is a pUC ori, which is operably linked to a repressing control sequence consisting of P22 P.sub.R ; (c) the product control sequence is P.sub.trc ; (d) a gene encoding a first repressor operably linked to a first inducible control sequence, wherein the first repressor is C2; and (e) a gene encoding a second repressor operably linked to a second inducible control sequence, wherein the second repressor is LacI.

22. The vaccine of claim 21, wherein the first activatible control sequence and and the second inducible control sequence are both araCP.sub.BAD.

23. The vaccine of claim 22, wherein the microorganism further comprises an inactivating deletion in the araCBAD operon and or the areE gene.

24. A method of inducing immunoprotection in a vertebrate comprising administering the vaccine of claim 19 to the vertebrate.

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L5: Entry 132 of 264 File: USPT Aug 24, 2004

US-PAT-NO: 6780405
DOCUMENT-IDENTIFIER: US 6780405 B1

TITLE: Regulated antigen delivery system (RADS)

DATE-ISSUED: August 24, 2004

INVENTOR-INFORMATION:

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NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Avant Immunotherapeutics, Inc.	Needham	MA			02

APPL-NO: 09/ 560539 [PALM]
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US-CL-CURRENT: 424/93.1; 424/200.1, 424/93.2, 424/93.4, 435/252.3, 435/320.1

FIELD-OF-SEARCH: 424/93.1, 424/93.2, 424/93.4, 424/200.1, 435/252.3, 435/320.1

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

Search Selected | Search ALL | Clear

	PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
☐	4190495	February 1980	Curtiss, III	
☐	4424065	January 1984	Langhoff et al.	
☐	4837151	June 1989	Stocker	
☐	4888170	December 1989	Curtiss, III	
☐	5015573	May 1991	Yarranton et al.	
☐	5028530	July 1991	Lai et al.	
☐	5278744	January 1994	Geboers et al.	
☐	5294441	March 1994	Curtiss, III	
	5389368	February 1995	Curtiss, III	

☐			
☐	5424065	June 1995	Curtiss, III
☐	5468485	November 1995	Curtiss, III
☐	5656488	August 1997	Curtiss, III
☐	5672345	September 1997	Curtiss, III
☐	5674746	October 1997	Morris
☐	5840483	November 1998	Curtiss, III
☐	5855879	January 1999	Curtiss III
☐	5855880	January 1999	Curtiss, III
☐	5888790	March 1999	Cahoon et al.
☐	6024961	February 2000	Curtiss, III

FOREIGN PATENT DOCUMENTS

FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	US-CL
9640947	June 1996	WO	

OTHER PUBLICATIONS

Bumann et al, Recdombinant live Salmonella spp. for human vaccination against heterologous pathogens, FEMS Immunology and Medical Microbiology, vol. 27, No. 4, Apr. 2000, pp. 357-364.
Wright et al, Dual-origin plasmids containing an amplifiable ColE1 ori; temperature-controlled expression of cloned genes, Gene, Elsevier Biomedical Press., Amsterdam, NL, vol. 49, No. 3, 1986, pp. 311-321.
Amann and Brosius, `ATG vectors` for regulated high-level expression of cloned genes in Escherichia coli, Gene 40:183-190 (1985).
Buchanan et al, Characterization of Antibody-Reactive Epitopes on the 65-Kilodalton Protein of Mycobacterium Leprae; Infect. Immun. 55:1000-1003 (1987).
Buxton et al., Heat-sensitive Lysis Mutants of Bacillus subtilis 168 Blocked at Three Different Stage of Peptidoglycan Synthesis; J. Gen. Microbiol. 120:283-293 (1980).
Curtiss and Kelly, Salmonella typhimurium Deletion Mutants Lacking Adenylate Cyclase and Cyclic AMP Receptor Protein are Avirulent and Immunogenic; Infec. Immun. 55:3035-3043 (1987).
Dul et al., Genetic Mapping of a Mutant Defective in D, L-Alanine Racemase in Bacillus subtilis 168; J. Bacteriol. 15:12121-214 (1973).
Galan et al., Cloning and characterization of the asd gene of Salmonella typhimurium: use in stable maintenance of recombinant plasmids in Salmonella vaccine strains; Gene, 94:29-35 (1990).
Jagusztyn-Krynicka, et al., Expression of streptococcus mutans Aspartate-Semialdehyde Dehydrogenase Gene Cloned into Plasmid pBR322; J. Gen. Microbiol. 128:1135-1145 (1982).
Kahn et al.,Plasmid Cloning Vehicles Derived from Plasmids ColE1, F, R6K, and RK2, Meth. Enzymol. 68:268-280 (1979).
Kleckner et al., Genetic Engineering in Vivo Using Translocatable Drug-resistance Elements; J. Mol. Biol. 116:125-159 (1977).
Nakayama et al., Construction of an ASD.sup.+ Expression-Cloning Vector: Stable Maintenance and High Level Expression of Cloned Genes in a Salmonella Vaccine Strain; Bio/Technology 6:693-697 (1988).
Ulmer et al.,DNA vaccines; Curr. Opin. Immunol. 8:531-536 (1996).

Umbarger, Amino Acid Biosynthesis and its Regulation, Ann. Rev. Biochem. 47:533 (1987).

ART-UNIT: 1645

PRIMARY-EXAMINER: Navarro; Mark

ATTY-AGENT-FIRM: Yankwich & Associates Yankwich; Leon R. Wesolowski; Michael R.

ABSTRACT:

We describe a regulated antigen delivery system (RADS) that has (a) a vector that includes (1) a gene encoding a desired gene product operably linked to a control sequence, (2) an origin of replication conferring vector replication using DNA polymerase III, and (3) an origin of replication conferring vector replication using using DNA polymerase I, where the second origin of replication is operably linked to to a control sequence that is repressible by a repressor. The RADS microorganism also has a gene encoding a repressor, operably linked to an activatible control sequence. The RADS described provide high levels of the desired gene product after repression of the high copy number origin of replication is lifted. The RADS are particularly useful as live bacterial vaccines. Also described is a delayed RADS system, in which there is a delay before the high copy number origin is expressed after the repression is lifted. The delayed RADS is also particularly useful for live bacterial vaccines. Also described are several control elements useful for these systems, as well as methods for providing immunity to a pathogen in a vertebrate immunized with the RADS microorganisms.

24 Claims, 23 Drawing figures

WEST Search History

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DATE: Monday, January 03, 2005

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		DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=AND	
☐	L1	letal near3 gene	1
☐	L2	lethal near3 gene	1553
☐	L3	L2 same (coli or salmonella or enterobacter$ or bacteri$)	400
☐	L4	L2 same (coli or salmonella or enterobacter$ or bacteri$ or procary$ or prokary$)	405
☐	L5	L4 and (immune or mucosal or iga or siga or vaccine or vaccination or therapeutic)	264
☐	L6	suicide.clm. and (cell or bacteria or bacterial or microbe or microbial or host or salmonella or coli or recombinant).clm.	309
☐	L7	L6 and (control or repressor or repression or promoter or inducible or induced or induction).clm.	122
☐	L8	L7 not l5	118
☐	L9	l8 and (temperature or nutrient or environment or environmentally or environmental or induction or induced or inducible).clm.	55
☐	L10	L9 and (internal and external)	15
☐	L11	environment$.clm. same induc$.clm.	899
☐	L12	L11 and suicid$.clm.	3

END OF SEARCH HISTORY

DOCUMENT-IDENTIFIER: US 5910438 A
TITLE: Cloning and/or sequencing vector

Brief Summary Text (13):
Pierce et al (Proc. Natl. Acad. Sci., vol 89. No. 6, 1992, pp. 2056-2060) describe a vector which comprises the lethal gene sac B from Bacillus amylolique-faciens, integrated into a plasmid derived from the bacteriophage P1 and under the control of a specific E. coli promoter.

Detailed Description Text (16):
Both in eucaryotes and in procaryotes, the cleavable topoisomerase II-DNA complex is the target of powerful therapeutic agents, including the antibiotics of the "quinolone" family, which act on the gyrase (bacterial topoisomerase II), and anticancer agents (acridines and epipodophyllotoxins), which act on the mammalian topoisomerase II. The therapeutic efficacy of the topoisomerase poisons is correlated with their ability to stabilize the cleavable complex.

DOCUMENT-IDENTIFIER: US 5916794 A
TITLE: Methods for inactivating target DNA and for detecting conformational change in a nucleic acid

Detailed Description Text (38):
This example shows that bacteria carrying the hybrid restriction enzymes gene can be forced to self-destruct by inducing the hybrid restriction enzymes. This example also provides proof of concept for potential use of hybrid restriction enzymes as therapeutic agents. Obviously, the hybrid restriction endonuclease genes could also be delivered into cells via a plasmid, virus, phage, or any other delivery vehicle that infects a particular type of bacterial or mammalian cells, including plant and animal cells; or the hybrid endonucleases could be introduced into cells by direct transfer via liposomes or by fusion to the translocating domains of bacterial toxins (Pastan et al., Biochem. Soc. Trans. 20:731-734 1992).

Detailed Description Text (40):
Bacteriophages have been shown to be effective in the treatment of experimental E. coli infection (10,11). More recently, bacteriophage was shown to prevent destruction of skin grafts by Pseudomonas aeruginosa (12). These bacteriophages can be engineered to carry the lethal hybrid endonuclease genes targeted against their hosts. These bacteriophages will be more effective in the destruction of the bacteria they infect. The present invention specifically includes this concept as well. The present invention also contemplates the delivery of other normal as well as mutant site-specific restriction enzymes using a similar approach.

Detailed Description Text (41):
One specific application of chimeric restriction enzymes is as therapeutics in the treatment of viral diseases caused by DNA viruses or retroviruses that replicate with a DNA intermediate. Chimeric restriction enzymes can be designed so that the DNA binding domain specifically targets viral-specific DNA sequences.

Detailed Description Text (42):
The main goal in treating viral infection is reducing viral load in infected cells and within a patient. Anti-viral drugs available today are generally toxic and have little specificity. Certain drugs are designed to inhibit a component of the virus's replicative machinery such as the enzymes thymidine kinase or reverse transcriptase. These agents do not destroy viral DNA. Other anti-viral agents act to promote the host's immune response so that infected cells are killed more efficiently. This results in non-specific destruction of both viral and host cell DNA.

Detailed Description Text (43):
At present there is a need for new therapeutic agents that specifically destroy viral DNA without destroying host cell DNA. Most viral DNA synthesis occurs within the cell's nucleus; thus it is important to generate therapeutic agents that can distinguish between viral and host cell DNA.

DOCUMENT-IDENTIFIER: US 6090562 A
** See image for Certificate of Correction **
TITLE: Recombinant clone selection system

Detailed Description Text (45):
Preferred lethal genes for E. coli include the active cytotoxic ccdB gene under the control of the lacP promoter, e.g., Bernard et al., Gene, 148: 71-74 (1994).

Detailed Description Text (54):
A particularly preferred binding pair useful in E. coli-based systems relies on the interaction of type 1 fimbriae of E. coli and bovine ribonuclease B (RNAse B). Most E. coli K-12 strains contain ca. 100-300 type 1 fimbriae at the cell surface, each fimbria having a width of ca. 7 nm and a length of 0.2 to 2 .mu.m, arranged pertinaciously around the cell. These fimbriae are adhesive organelles important for successful bacterial recognition and colonization of specific host tissues. A single fimbria consists of ca. 1,000 repeating subunits of mostly a single polypeptide (FimA) having a molecular mass of ca. 17 kilodaltons (kDa). The minor subunit (fimH) mediates specific binding to D-mannosyl residues e.g., Klemm, P., and Krogfelt, K. A. in P. Klemm (ed.), Fimbriae, Adhesion, Genetics, Biogenesis and Vaccines, p 9-26, CRC Press, Boca Raton, Fla. (1994). The fact that type 1 fimbriae exhibit high affinity for D-mannosyl residues suggested the use of bovine ribonuclease B as the complementary member of the binding pair. Bovine ribonclease B is a glycoprotein with a molecular mass of 15.5 kDa which consists of 124 amino acids with a unique glycosylation site including five glycoforms with oligosaccharides of five to nine mannose residues, e.g., D. Fu, L. Chen, and R. A. O'Neill Carbohydr. Res. 261: 173-186 (1994). The well known ability of RNAse B to "stick" to hydrophobic substrates such as glass and polystyrene suggested that suitably patterned substrates with adsorbed RNAse B could be used to capture single E. coli cells (1.times.3 .mu.m).

Other Reference Publication (41):
Griffiths et al., "Somatic mutation and the maturation of immune response to 2-phenyl oxazolone," Nature 312:271-275 (Nov. 15, 1984).

DOCUMENT-IDENTIFIER: US 4888280 A
**** See image for Certificate of Correction ****
TITLE: Hybrid proteins produced by an ultrahigh prokaryotic expression

Brief Summary Text (3):
The lambda P.sub.L promoter has been successfully used in conjunction with a CI857 temperature-sensitive lambda repressor. This allows for low level expression of the cloned product during E. coli growth at 30.degree. C. Once substantial cell density is established, the cloned gene can be derepressed by growth at 42.degree. C. This method has been used in the expression of gene products lethal to the host cells. Several investigators have reported expression levels of 4% (Waldman, A. S., Haensslein, E., and Milman, G. [1983] J. Bio. Chem. 258: 11571-11575); 7% (Yoakum, G. H., Yeung, A. T., Mattes, W. B., and Grossman, L. [1982] PNAS 79: 1766-1770; Derom, C., Gheysen, D., and Fiers, W., [1982] Gene, 17: 45-54); and 13% (Oehrnichen, R., Klock, G., Altschmid, L., and Hillen, W. [1984] EMBO J. 3: 539-543) using the P.sub.L promoter under thermolabile repressor control.

Drawing Description Text (66):
Protein A is widely used as an immunoabsorbent in a variety of diagnostic and basic research test systems. See U.S. Pat. No. 4,322,274. Recent interest in applications of protein A has centered around its possible clinical use in anticancer treatment. Sensitized peripheral blood lymphocytes, normally responsible for cytotoxicity of tumor cells, are hypothesized to be inhibited in this function by serum blocking factors which are presumed to consist of specific antigens, antibodies, antiglobulins, and immune complexes. See Barnes, B. C. (1981) Cancer Bull. 33: 278. These "blocking" factors can be removed from sera of tumor-bearers by absorption to S. aureus, Cowan I cells which contain protein A, and thus allow cell-mediated tumor cell toxicity to proceed in in vitro test systems. See Steele, G., Ankerst, J., and Sjogren, H. (1974) Int. J. Cancer 14: 83. Protein A also activates polyclonal antibody synthesis independent of its IgG binding activity. See Sjodahl, J. and Moller, G. (1979) Scand. J. Immunol. 10: 593.

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☐ 1. Document ID: US 20030183306 A1

Using default format because multiple data bases are involved.

L12: Entry 1 of 3 File: PGPB Oct 2, 2003

PGPUB-DOCUMENT-NUMBER: 20030183306
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20030183306 A1

TITLE: Selected processing for non-equilibrium light alloys and products

PUBLICATION-DATE: October 2, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Hehmann, Franz	Osnabrueck		DE	
Weidemann, Michael	Isernhagen		DE	

US-CL-CURRENT: 148/404; 148/420, 148/437

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw D

☐ 2. Document ID: US 5681745 A

L12: Entry 2 of 3 File: USPT Oct 28, 1997

DOCUMENT-IDENTIFIER: US 5681745 A
** See image for Certificate of Correction **
TITLE: Biotin-binding containment systems

CLAIMS:

4. The genetic containment system of claim 1 wherein the cassette comprises a regulatable promoter and a suicide gene.

5. The containment system of claim 4 wherein the regulatable promoter is functionally linked to said suicide gene.

6. The containment system of claim 4 wherein the suicide gene is selected from the group consisting of E. coli gef genes, Serratia marcescens and Staphylococcus aureus endonucleases, Serratia liquefaciens phospholipase A, Bacillus subtilis sacB gene, lysis genes from bacteriophages and combinations thereof.

7. The containment system of claim 4 wherein the suicide gene encodes said biotin-binding component.

24. A genetic containment system comprised of a cassette that encodes a suicide gene and a marker gene that encodes a biotin-binding protein.

26. A vector that contains a genetic containment system comprised of a suicide gene that encodes a biotin-binding protein whose expression is regulated by a transcriptional effector encoded within said vector wherein expression of said transcriptional effector is regulated by a physiological condition.

37. A recombinant cell containing a genetic containment system comprised of a suicide cassette that encodes a biotin-binding protein.

41. A genetic containment system comprising a suicide gene encoding a biotin-binding protein functionally linked to an inducible promoter, a suicide control gene encoding a heterologous polymerase functionally linked to a repressible promoter regulated by a repressor, and a polymerase control gene functionally linked to a constitutive promoter.

42. The genetic containment system of claim 41 wherein the suicide gene encodes streptavidin, avidin or mutants.

50. The genetic containment system of claim 49 wherein the another inducible promoter that is activated or repressed in response to a change of an environmental condition.

52. A genetic containment system comprising a suicide gene encoding a biotin-binding protein functionally linked to an inducible promoter, a suicide control gene encoding a polymerase functionally linked to a repressible promoter, a polymerase control gene functionally linked to a constitutive promoter, and a gene that encodes said repressor functionally linked to another inducible promoter that responds to an environmental condition.

☐ 3. Document ID: US 5679533 A

L12: Entry 3 of 3 File: USPT Oct 21, 1997

DOCUMENT-IDENTIFIER: US 5679533 A
TITLE: Biotin-binding containment systems

CLAIMS:

1. A method for selectively killing a recombinant organism comprising the steps of:

a) providing a cell that contains a suicide cassette comprised of a suicide gene that encodes a biotin-binding protein functionally linked to a promoter whose activity is regulated by a transcriptional effector; and

b) stimulating said transcriptional effector.

10. A method for selectively killing a recombinant organism comprising the steps of: of:

a) providing a cell that contains a cassette comprised of a suicide gene that encodes a biotin-binding protein functionally linked to an inducible promoter, a suicide control gene encoding a heterologous polymerase functionally linked to a repressible promoter regulated by a repressor, and a polymerase control gene functionally linked to a constitutive promoter; and

b) stimulating said inducible promoter.

11. The method of claim 10 wherein the suicide gene encodes streptavidin, avidin mutants thereof.

19. The method of claim 18 wherein said second inducible promoter is activated or repressed in response to a change of an environmental condition.

21. A method for selectively killing a recombinant organism comprising the steps of:

a) providing a cell that contains a cassette comprised of a suicide gene encoding a biotin-binding protein functionally linked to an inducible promoter, a suicide control gene encoding a polymerase functionally linked to a repressible promoter, a polymerase control gene functionally linked to a constitutive promoter, and a gene that encodes said repressor functionally linked to second inducible promoter that responds to an environmental condition; and

b) stimulating said inducible promoter.

Detailed Description Text (59):
Cai, Y., and Wolk, P. C. (1990) Use of a conditionally lethal gene in Anabaena sp. strain PCC7120 to select for double recombinants and to entrap insertion sequences. J. Bacteriol 172:3138-3145.

First Hit Fwd Refs

L5: Entry 143 of 264 File: USPT Aug 26, 2003

US-PAT-NO: 6610529
DOCUMENT-IDENTIFIER: US 6610529 B1

TITLE: Recombinant bacterial system with environmentally limited viability

DATE-ISSUED: August 26, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Curtiss, III; Roy	St. Louis	MO		
Tinge; Steven A.	Belleville	IL		

US-CL-CURRENT: 435/252.3; 424/257.1, 424/258.1, 424/93.1, 424/93.2, 424/93.48, 435/442, 435/471, 435/481, 435/69.1

CLAIMS:

We claim:

1. An isolated microbial cell comprising a genetically engineered Environmentally Limited Viability System, wherein the cell is viable in a permissive environment and non-viable or temporarily viable in a non-permissive permissive environment, the system comprising (a) an essential gene located on an extrachromosomal vector, wherein expression of the essential gene in the cell is essential to the viability of the cell, the essential gene is expressed expressed when the cell is in the permissive environment and is not expressed or temporarily expressed when the cell is in the non-permissive environment, and wherein the essential gene is a copy of a wild-type gene of the microbial cell; and (b) a lethal gene located on an extrachromosomal vector, wherein the expression of the lethal gene is regulated by an expression product of a regulatory gene located on the chromosome of the cell, and wherein expression of the lethal gene is lethal to the cell and the lethal gene is expressed when the cell is in the non-permissive environment but not when the cell is in the permissive environment, wherein the wild-type essential gene is inactivated in the cell, and wherein the essential gene is essential for metabolism, growth, cell wall integrity, or cell membrane integrity of the cell.

2. The cell of claim 1 wherein the cell grows in the permissive environment and and dies in the non-permissive environment.

3. The cell of claim 1 wherein the permissive environment comprises an environment containing a nutrient required to maintain expression of the essential gene, prevent expression of the lethal gene, or both, and the non-permissive environment comprises an environment lacking the nutrient.

4. The cell of claim 1 wherein the permissive environment is inside a warm-blooded animal and the non-permissive environment is outside a warm-blooded animal.

5. The cell of claim 1, wherein the essential gene comprises the asd gene operatively linked to araC-P.sub.BAD.

6. The cell of claim 1 wherein the expression product of the regulatory gene inhibits expression of the lethal gene, and wherein the regulatory gene is expressed or active only in the permissive environment.

7. The cell of claim 1 wherein the vector has two lethal genes.

8. The cell of claim 1 wherein the cell is a gram-negative bacterium.

9. The cell of claim 8 wherein the gram-negative bacterium is an enteric bacterium.

10. The cell of claim 9 wherein the genus of the enteric bacterium is selected from the group consisting of Escherichia and Salmonella.

11. The cell of claim 1, wherein expression of the essential gene is regulated by an expression product of a regulatory gene, wherein the expression product of the regulatory gene inhibits expression of the essential gene and is expressed or active only in the non-permissive environment, and wherein the essential gene comprises the asd gene operatively linked to araC-P.sub.BAD.

12. The cell of claim 1 further comprising an expression gene wherein the expression gene encodes a desired expression product.

13. The cell of claim 1 wherein the cell is temporarily viable in the non-permissive environment.

14. The cell of claim 1 further comprising a transfer vector.

15. The cell of claim 1 wherein the lethal gene is a gene required for excision of a prophage, wherein the prophage is in the chromosome of the cell, and wherein excision of the prophage causes lysis of the cell.

16. The cell of claim 1 wherein the essential gene, the lethal gene, or both have engineered expression.

US-PAT-NO: 5679533
DOCUMENT-IDENTIFIER: US 5679533 A

TITLE: Biotin-binding containment systems

DATE-ISSUED: October 21, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Szafranski; Przemyslaw	Boston	MA		
Mello; Charlene M.	Rochester	MA		
Sano; Takeshi	Boston	MA		
Marx; Kenneth A.	Francestown	NH		
Cantor; Charles R.	Boston	MA		
Kaplan; David L.	Stow	MA		
Smith; Cassandra L.	Boston	MA		

US-CL-CURRENT: 435/7.2; 435/262, 435/262.5, 435/320.1, 435/6, 435/7.32, 435/7.37

CLAIMS:

We claim:

1. A method for selectively killing a recombinant organism comprising the steps steps of:

a) providing a cell that contains a suicide cassette comprised of a suicide gene that encodes a biotin-binding protein functionally linked to a promoter whose activity is regulated by a transcriptional effector; and

b) stimulating said transcriptional effector.

2. The method of claim 1 wherein the cell is a genetically engineered microorganism.

3. The method of claim 2 wherein the genetically engineered microorganism is used for bioremediation.

4. The method of claim 1 wherein the biotin-binding protein is streptavidin or avidin.

5. The method of claim 1 wherein the promoter is a T7 promoter and the transcriptional effector is a T7 RNA polymerase.

6. The method of claim 1 wherein the promoter is a lac promoter or part thereof.

7. The method of claim 1 wherein the addition of an extrinsic agent stimulates transcription from said transcriptional effector or inactivates a transcriptional repressor of said transcriptional effector.

8. The method of claim 7 wherein the extrinsic agent is IPTG or an analog thereof.

9. The method of claim 1 wherein the cell constituitively expresses an inhibitor of the transcriptional effector.

10. A method for selectively killing a recombinant organism comprising the steps of:

a) providing a cell that contains a cassette comprised of a suicide gene that encodes a biotin-binding protein functionally linked to an inducible promoter, a suicide control gene encoding a heterologous polymerase functionally linked to a repressible promoter regulated by a repressor, and a polymerase control gene functionally linked to a constitutive promoter; and

b) stimulating said inducible promoter.

11. The method of claim 10 wherein the suicide gene encodes streptavidin, avidin mutants thereof.

12. The method of claim 10 wherein the inducible promoter is a bacteriophage .lambda., SP6, T3 or T7 promoter.

13. The method of claim 10 wherein the heterologous polymerase is a bacteriophage, SP6, T3 or T7 polymerase.

14. The method of claim 10 wherein the repressor is a lactose, galactose or tryptophan repressor protein.

15. The method of claim 10 wherein the repressible promoter is P.sub.tac, P.sub.lac, P.sub.trp, P.sub.gal or P.sub.phoA.

16. The method of claim 10 wherein the polymerase control gene encodes a lysozyme.

17. The method of claim 10 wherein the constitutive promoter is P.sub.m.

18. The method of claim 10 further comprising a gene encoding the repressor protein that is functionally linked to a second inducible promoter.

19. The method of claim 18 wherein said second inducible promoter is activated or repressed in response to a change of an environmental condition.

20. The method of claim 19 wherein the environmental condition is the change in in concentration of a chemical, metal, radiation or nutrient or change in pH.

21. A method for selectively killing a recombinant organism comprising the steps of:

a) providing a cell that contains a cassette comprised of a suicide gene encoding a biotin-binding protein functionally linked to an inducible promoter, promoter, a suicide control gene encoding a polymerase functionally linked to a a repressible promoter, a polymerase control gene functionally linked to a constitutive promoter, and a gene that encodes said repressor functionally

linked to second inducible promoter that responds to an environmental condition; and

b) stimulating said inducible promoter.